MODIFICATION BY PRACTOLOL OF CHANGES IN CA METABOLISM INDUCED BY HYPOXIA AND BY ISOPROTERENOL

by

CATHERINE CHEUK-YING PANG

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ABSTRACT

It is well known that hypoxia causes a rapid reduction of contractility in the mammalian myocardium. Since at least two Ca pools are essential for the maintenance of contractility, one purpose of this investigation was to determine if hypoxia caused changes in Ca exchange or distribution in these pools. Hypoxia was produced in modified Langendorff kitten heart preparations by perfusion with modified Krebs-Henseleit solution (K-H) equilibrated with 5% O_2, 5% CO_2 and 90% N_2. By perfusion with a Ca-free K-H solution, it was found that 3 min of hypoxia resulted in a significant reduction of a Ca washed out of a pool, Ca_{II} which is essential for the maintenance of contractile force in the heart. From reperfusion of hearts depleted of Ca, it was found that Ca taken up by a small, superficial pool, Ca_1, was also significantly reduced after 6 min of hypoxia. Ca_1 represents a Ca pool that may mediate the release of Ca from Ca_{II}. Thus, the reduction of contractility in early hypoxia was associated with reduction of the Ca contents of two Ca pools, Ca_1 and Ca_{II}. Ca-free perfusion studies have also shown that 10 min hypoxia resulted in a small increase of the quantity of Ca washed out of a pool, Ca_{III}, which may represent Ca binding sites involved in Ca sequestration for relaxation, that is, the Ca contained in the mitochondria and the sarcoplasmic reticulum. The Ca content in the mitochondria was found by subcellular fractionation studies to be slightly increased after 10 min of hypoxia. Although the total quantity of Ca washed out of the hearts after Ca-free perfusion was not changed by 10 min hypoxia, there was a significant increase in the quantity of tightly bound Ca remaining in the heart. This residual Ca

probably represents Ca not involved with coupling excitation to contraction.

The effect of practolol, a β_1 adrenergic blocking agent, on Ca exchange in hypoxic hearts was also investigated. Practolol (10^{-5}M) prevented the reduction in Ca_{II} and the increase in Ca_{III} contents associated with hypoxia. The drug also reduced the hypoxia-induced reduction in Ca_1 content. The increase in residual, non-exchangeable Ca after hypoxia was however not prevented by practolol. Thus, practolol prevented changes in the Ca pools that are important in coupling excitation to contraction. Practolol, however, neither prevented the reduction of contractility associated with hypoxia nor increased the rate of restoration of contractility upon perfusion with a normoxic K-H solution. Thus, the reduction of contractility associated with hypoxia may have been caused by at least two factors, namely, changes in the Ca involved in excitation-contraction coupling and reduction in the supply of high energy phosphates. Improvement of contractility probably depends on amelioration of both factors.

The effect of isoproterenol on Ca exchange was compared with that of hypoxia since both treatments cause different effects on contractility but were reported to cause similar cellular damage presumably due to an accumulation of Ca in the mitochondria. Isoproterenol was found to significantly increase the Ca content of Ca_{II} which may be related to the positive inotropic effect of this drug. Although practolol prevented the positive inotropic effect of isoproterenol, practolol did not prevent the isoproterenol induced increase of Ca_{II} content. Practolol, however, decreased the content of Ca in Ca_1 and by this mechanism may have prevented the increase of contractility caused by isoproterenol. Unlike the effect of hypoxia, isoproterenol was found to reduce both the content of Ca in Ca_{III} as well as the content of

Ca in the mitochondria. The effect of isoproterenol was thus found in this investigation to be quite different from that of hypoxia.

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INTRODUCTION

I. Historical Background.

A. Definition of ischemic heart disease.

> "When you examine a man for illness in his cardia, he has pains in his arm, in his breast, on the side of his cardia; it is said thereof: this is the w3d-illness. Then you shall say thereof: it is something which entered his mouth; it is death which approaches him."
>
> Ebbell in "The Papyrus Ebers"

The above quotation from Ebbell's English translation of the famous "Papyrus Ebers" describes possibly the precordial pain that is associated with coronary heart disease and the eventual fatal outcome of the disease in ancient Egyptian time. Pathological examination of coronary arteries of a female Egyptian mummy who died when she was about 50 years old, of the 21st dynasty (about 1000 B.C.) was made by A.R. Long in 1931. Long was able to detect the presence of fibrous thickening and calcification of the coronary arteries. Thus, the existence of ischemic heart disease in ancient times was established.

It is generally accepted that ischemia refers to a condition in which the flow of arterial blood is insufficient to provide sufficient oxygen to the myocardium, thus shifting cellular respiration from aerobic to the anaerobic form (Jennings, 1970). In contrast, hypoxia is a condition in which blood or perfusate flow is not compromised, but contains insufficient oxygen for normal muscle function. If ischemia is prolonged to result in cell death, a myocardial infarct is present (Jennings, 1970). It is now recognized that in myocardial infarct, there exists areas of dead cells with little or no coronary blood flow, areas which are well-

perfused and infarcted myocardium (Cox et al., 1968; Jennings, 1970). Therapy is aimed at increasing the energy supply to, or reducing energy demand of ischemic or mildly ischemic areas, thus reducing cell death.

Although a description of clinical syndromes resembling coronary artery disease was made as early as the sixteenth century, the disease was not established as a separate entity at that time. The first description of coronary calcification was made by Bellini in 1683. Bellini reported the presence of a 'stone' in the coronary arteries of a patient who died of a condition similar to the clinical picture of coronary artery disease. In 1768, Heberden introduced the name and in 1772 published the clinical symptoms of "Angina Pectoris". The next important contribution was made by Jenner who correctly diagnosed Hunter's disease of the coronary arteries through observation of Hunter's disease symptoms during a chance meeting with Hunter at a spa (Parry 1799). At necropsy, Hunter's coronary arteries were indeed found to be calcified as Jenner had predicted. Jenner did not tell Hunter of his condition while he was alive fearing that knowledge of a disease of the coronary arteries might have prevented his dear friend and teacher of the hopes of a recovery.

The earliest study on the effect of coronary ligation on myocardial function was performed in 1698 by Chirac who observed that one minute after tying the coronary artery of a dog, the heart stopped beating. It was almost 150 years before Erichsen in 1842 recognized coronary obstruction as occurring frequently in association with sudden death in man, and ligated the coronary arteries near their origin in dogs and rabbits. Erichsen reported cardiac standstill in all of the animals

studied and concluded that any disease, such as "ossification" of the coronary arteries, that interferes with the flow of blood though the vessels may result in sudden death. One of the most conspicuous studies of the nineteenth century was made by the famous pathologist Cohnheim in 1881 who stated that many conditions, such as fibrous myocarditis or heart aneurysms are due to coronary obstruction and that oxygen lack is responsible for the myocardial damage. Cohnheim however, believed that coronary arteries were end arteries; that is, collaterals did not exist, and thus concluded that occlusion must always lead to sudden death (Cohnheim and Schulthess-Rechberg, 1881). This view was unfortunately accepted by most clinicians at the time. In 1893, Porter reported that ligation of the coronary arteries was followed by infarction of the muscles supplied by those arteries. Porter further demonstrated in 1894 that ligation of a major coronary artery was not necessarily fatal. Hirsch and Spalteholz (1907) ligated the descending branch of the left coronary artery in dogs and apes without incidence of sudden death among the animals studied. On autopsy, the authors found a few, small scattered areas of necrosis in the wall of the left ventricle, considerably less in extent than that supplied by the ligated vessel. The authors concluded the existence of physiological or functional anastomoses in the coronary arteries in addition to the anatomic anastomoses previously described by Merkel in 1907.

The next advance was made by Herrick in 1912 who described the syndrome of acute myocardial infarction and differentiated this syndrome from angina pectoris which does not necessarily involve permanent myocardial damage. In 1928, Keefer and Resnik suggested that angina pectoris was

due to anoxemia of the myocardium and thereby initiated a new era of investigation, because for decades, nearly all disturbances of the heart muscle were attributed to impairments of coronary blood flow, ranging from hypothetical coronary spasms to sclerotic stenosis and thrombolic occlusion and not simply to a lack of oxygen supply to the heart.

If localized tissue hypoxia due to coronary occlusion is responsible for muscle cell death, then it was reasoned, total tissue hypoxia caused by breathing a hypoxic or an anoxic gas mixture should produce the same effects. However, the effect on the heart of diminished concentrations of oxygen in the inspired air was not studied until the early twentieth century. In 1920, Barcroft reported that the effect of acute anoxaemia on man is transient and could be reversed with time and that chronic anoxaemia caused fatigue and irritability. Complete deprivation of oxygen, on the other hand caused cardiac standstill. Green and Gilbert (1921a, 1921b) studied the effect of oxygen deprivation in young men and dogs by rebreathing experiments and described a decrease in the amplitude of the T wave in the early stages of deprivation. Gremels and Starling (1926) reported that in the heart-lung preparation anoxemia resulted in dilatation of the heart followed by heart failure. Luft (1937) reported necrosis in the papillary muscle and the left ventricular wall of hearts of guinea pigs which had been subjected to low atmospheric pressure (230-330 mm Hg) for 120 to 180 hours. Levy et al., (1938) compared the effect of oxygen lack to coronary occlusion and found that administration of an atmosphere containing 12% oxygen to patients with coronary insufficiency caused pain together with reversible electrocardiographic changes similar

to those following coronary occlusion. However, these treatments produced total tissue hypoxia which may have obscured the effects of low oxygen tension on the heart. Therefore, while inspiration of hypoxic gas mixtures will produce myocardial oxygen deficiency the procedure is not a practical method for the study of myocardial ischemia.

The effect of ischemia or hypoxia on myocardial electrolyte levels was first studied in 1939 by Hastings et al. who reported that temporary occlusion of a major coronary artery produced an increase of myocardial Na, Cl and water. Following Hasting's report, Lowry et al., (1942) studied the effect of anoxemia on myocardial electrolyte levels in the heart-lung preparation. These investigators found that a gas mixture containing 5% CO_2 and 95% N_2 caused instantaneous heart failure with only small changes in circulating electrolyte levels. However, when a hypoxic gas mixture containing 2% O_2 and 98% N_2 was used, the heart continued to beat for an hour before fibrillation occured. There was an increase of water, Na, and Cl together with a decrease of K in these hearts. The effect of ischemia on myocardial electrolyte levels was also studied by Shen and Jennings (1972 a) who reported increases in tissue Ca, Na and water together with reductions in tissue Mg and K in cells subjected to 40 min ischemia followed by 20 min reperfusion. Lie et al., (1975) studied the effect of coronary ligation on myocardial cationic electrolyte levels and reported depressions of K/Na and Mg/Ca in infarcted area and areas bordering the infarct. The investigators suggested that these lowered electrolyte ratios might be used as sensitive indices of myocardial ischemia.

B. Contractility in ischemia and hypoxia.

It has been known since 1935 that one of the first signs of myo-

cardial ischemia was a decrease in myocardial contractile activity. Prior to this time, a suitable myograph was not available to record changes in muscle length and not artefacts due to position changes and vibrations of the beating ventricle (Tennant and Wiggers, 1935). Tennent and Wiggers (1935) reported that 1 min after coronary occlusion, the ischemic region of the heart ceased to contract. Prinzmetal et al., (1949), by means of slow motion color pictures, found that areas of the heart supplied by the occluded vessel may cease to contract in as little as 5 sec after coronary ligation. At this time, the ischemic myocardium bulges outwards during systole. Furchgott and de Gubareff (1958), by means of an optical lever for recording contractile activity, reported a decrease in contractile strength of isolated atria exposed to anoxia. Tatooles and Randoll (1961) and Schelbert et al., (1971) using strain gauge arches observed a reduction of contractile force in ischemic area of the myocardium, when compared to the control. Tyberg et al., (1970) and Pirzada et al., (1975) reported immediate reduction of contractile force of isolated papillary muscle and trabecular muscle, respectively, when exposed to an anoxic gas mixture.

a. The effect of oxygen lack on electrical activity

The early failure of effective contraction in the ischemic region of the myocardium could theoretically be due to inability of the muscle to generate or propagate an action potential. However, this has not been found to be the case. After ligation of the anterior descending branch of the left coronary artery and stopping the heart by vagal stimulation. Tennant and Wiggers (1935) were able to cause the heart to contract again using a stimulus which had been just adaquate to excite the normal ven-

tricle. Prinzmetal et al., (1949) also demonstrated that the ischemic myocardium retains its ability to conduct an impulse for contraction since the minimum strength of the electrical stimulus which was effective in producing a ventricular extrasystole remained the same before and up to 19 min after coronary ligation. Thus, the threshold to electrical stimulation in the ischemic myocardium appears to be normal in early ischemia. Furthermore, electrical activity also persists in the anoxic and ischemic myocardium when contractility has fallen drastically. Sayen et al., (1958) reported that there was no electrocardiographic changes occurring 15-25 sec after coronary occlusion when late systolic bulging of the ischemic muscle occurred. Redo and Porter (1959) reported that electrical activities persisted long after mechanical activity had ceased in ischemic hearts.

b. The effect of oxygen lack on contractile proteins

Another possible cause for the early reduction of contractility in hypoxia and ischemia could be due to structural changes of the cardiac contractile proteins, resulting in a change of the number or nature of the interactions between actin and myosin filaments. Most investigators, believe that no irreversible damage to cardiac actin (Katz and Maxwell, 1964), myosin (Bárány et al., 1964), or myofibrils (Brown et al., 1959; Alpert and Gorden 1962) could be attributed to prolonged ischemia. Although ischemia has not been found to cause any permanent damage to the heart's contractile proteins, it can still theorectically cause reversible conformational changes of these contractile proteins in situ which are subsequently reversed during their isolation. Until it is possible to characterize the physicochemical state of the contractile proteins of the in-

tact heart, this question remains unanswered.

c. The effect of oxygen lack on energy supply and utilization

One of the first effects of oxygen deprivation is to cause a significant reduction of myocardial oxygen tension. Sayen et al., (1958) reported that following coronary ligation, myocardial oxygen falls to 10% of its control value within 30 sec. It would be expected that oxidative metabolism and also high energy phosphate stores under such conditions would be greatly reduced. The levels of adenosine triphosphate (ATP) and creatine phosphate (CP) have been reported to be reduced in heart muscles during anoxia (Furchgott and de Gubareff, 1958; Michal et al., 1959) and ischemia (Degenring, 1975). Since these compounds are the energy source for muscular contraction, it is to be expected that the reduction of ATP or CP stores, if occurs early in hypoxia or ischemia may bring about a reduction of myocardial contractility. Indeed, several investigators have reported a reduction of ATP or CP levels within 1/2 to 1 min after the onset of myocardial ischemia (Braasch, 1968); hypoxia (Scheuer et al., 1968; Dhalla et al., 1972), and acute asphyxia (Furchgott, 1961; Feinstein, 1962) when contractility was reduced.

d. The effect of oxygen lack on myocardial excitation-contraction coupling

Another primary factor which may be involved in early reduction of myocardial contractility associated with ischemia or hypoxia is a decrease of Ca release from cardiac sarcoplasmic reticulum to couple excitation to contraction. The importance of Ca for myocardial contraction has been realized since 1882 when Ringer reported that Ca was required for the heart to maintain its ability to contract and

that Ca would restore contractility in hearts bathed in saline solution. Heilbrunn and Wiercinski (1947) established the role of Ca in muscular contraction by showing that intracellular injection of Ca into isolated heart muscle fibers initiated an immediate contraction. The studies of Winegrad (1961) and Niedergerke (1963b) demonstrated that Ca couples excitation to contraction since the influx of Ca into myocardial cells during excitation was associated with contraction. Moreover, increment in Ca influx brought about either by increasing the frequency of stimulation or by elevation of external Ca concentration was found to result in increase strength of contraction. Niedergerke (1963 a) suggested that Ca from the extracellular space (Ca_1) entered the cell in combination with a carrier molecule and was then released as an active form or ionic form to activate the contractile mechanism. Afterwards it was stored in an inactive form until finally expelled from the cell by an active process since the electrochemical gradient favors the accumulation of Ca in the cell. Ca_1 may represent the transmembrane exchange and supply pool of Ca proposed by Tritthart et al., (1973) which is believed to be equivalent to the slow, inward Ca current (Beeler and Reuter, 1970 a,b,c; Ochi and Trautwein, 1971). By voltage clamp technique, it was found that the threshold for the slow, inward Ca current (approximately -35 mV) coincides with the threshold for tension development (Beeler and Reuter, 1970 a,b). The above evidence therefore suggests that Ca current might couple excitation to contraction in cardiac muscle.

Pharmacological agents such as Ni, Co, Mn, D600 and verapamil which selectively inhibits slow, inward Ca current have been found to uncouple excitation from contraction (Kohlhardt et al., 1973). Furthermore, La,

which blocks transcellular Ca movement without penetrating the cell membrane uncouples excitation to contraction in mammalian cardiac tissue (Mascher, 1970; Sanborn and Langer, 1970; Rich and Langer, 1975). The results therefore showed that in the absence of transmembrane Ca influx excitation failed to cause contraction of cardiac cells.

The magnitude of cardiac contraction, however, is not a simple correlate to the size of the Ca current. Beeler and Reuter (1970 c) have found that following a quiescent period, the slow, inward current was fully activated with the first depolarization and did not further increase during subsequent depolarizations despite a steady increase (staircase) of contractile force over five to eight depolarizations. The results thus suggest the existence of an intracellular pool of Ca which supplies the Ca released to the myofilaments upon repetitive depolarizations. Further evidence to support the existence of an intracellular supply of Ca is given by voltage clamps studies under conditions of Na-free perfusion (Beeler and Reuter, 1970 c). Tension development after a period of quiescence was found to be maximal with the first depolarization even when there was no discernible increase in slow inward current. It therefore appears that Na-free perfusion also augments Ca release from an intracellular store for activation of the contractile mechanism. Moveover, in mammalian cardiac muscle, the transmembrane inward Ca current that occurs during the plateau of the action potential seems insufficient to activiate contraction (Bassingthwaighte and Reuter, 1972). Additional Ca is therefore believed to be released from internal stores for contraction.

The need for Ca influx for contraction suggests that the influx of Ca may induce subsequent release of Ca from an intracellular pool. The concept of "trigger Ca" or a regenerative release of Ca has been proposed basing on studies in skinned skeletal muscle fibres (Endo et al., 1970; Ford and Podolsky, 1970). The small trans-sarcolemmal influx of Ca is supposed to trigger the release of Ca from internal stores, presumably located in the sarcoplasmic reticulum. These studies were later criticized to be not physiological since sarcoplasmic reticulum was first loaded with high concentrations of Ca which resulted in dilation of sarcoplasmic reticulum (Rich and Langer, 1975). Regenerative release of Ca in the skeletal muscle was also found to be unlikely since tension declines slowly in the absence of external Ca (Langer, 1973; Rich and Langer, 1975). This suggests that external Ca plays a small role in excitation-contraction coupling of the skeletal muscle.

Evidence against triggered release of Ca in the skeletal muscle does not necessarily mean lack of such a mechanism in the cardiac muscle. Ultrastructurally, the diameter of transverse tubular system (T system) in heart is about five times of that in skeletal muscle (Fawcett and McNutt, 1969). Furthermore myocardial T system is lined with a layer of basement membrane material composed of negatively-charged mucopolysaccharide which has a strong affinity for Ca (Scott, 1968). In contrast, although the basement membrane is present on the sarcolemma of mammalian skeletal muscle, it is absent in the invaginating T-system lumina (Fawcett and McNutt, 1969). Thus, skeletal muscle basement membrane is less efficient in Ca binding than myocardial basement membrane. Perfusion studies using isolated kitten hearts (Bailey and Dresel, 1968) or guinea-pig intra-

ventricular septa (Rich and Langer, 1975) showed a rapid decline of contractile force when external Ca was removed. These results indicate that, in contrast to skeletal muscle, cardiac muscle depends on external Ca for contractile activities. In isolated cardiac cells with disrupted sarcolemma (Fabiato and Fabiato, 1972) and single skinned cardiac cell Fabiato and Fabiato, 1975), transient contractions were induced by a concentration of free Ca much lower than that required to activate the myofilaments directly. This suggests that trigger Ca may play a physiological role in excitation-contraction coupling of heart muscle.

It is well known that the two major intracellular storage sites of Ca are the sarcoplasmic reticulum and the mitochondria. In skeletal muscle, it is believed that the sarcoplasmic reticulum regulates the intracellular concentration of Ca for contraction and relaxation (Weber et al., 1963, Sandow, 1965; Ebashi and Endo, 1968). The role of the sarcoplasmic reticulum in the control of contraction and relaxation of the heart has been suggested (Carsten, 1964; Katz and Repke, 1967, Weber et al., 1967; Pretorius et al., 1969 and Schwartz, 1971). It is believed that cardiac sarcoplasmic reticulum has the rate and capacity for Ca binding to account for muscle relaxation. Moreover, pre-treatment with caffeine, which inhibits Ca sequestration by the sarcoplasmic reticulum (Weber and Herz, 1968; Fabiato and Fabiato, 1975) or with deoxycholate which destroys the sarcoplasmic reticulum (Hellam and Podolsky, 1969), were found to inhibit cyclic contractions of skinned cardiac cells. Therefore it appears that the sarcoplasmic reticulum played a major role in Ca binding and release. Bloom et al., (1974) working with mechanically disaggregated heart muscle has also presented similar evidence that the

sarcoplasmic reticulum is required for cyclic contraction and relaxation. The exact role of heart mitochondria in the control of Ca for contraction and relaxation is less certain. Fabiato and Fabiato (1975) reported that neither azide which inhibits oxidative phosphorylation and energy-linked transport of Ca by mitochondria nor ruthenium red which inhibits passive binding of Ca by mitochondria has been found to modify cyclic contractions of skinned cardiac cell. Thus, the mitochondria are believed to play little, if any, role in the initiation of cyclic contractions. Some investigators however, believe that both the sarcoplasmic reticulum and mitochondria participate in the regulation of Ca for coupling excitation to contraction in the heart (Lehninger, 1974; Carafoli, 1975).

Since the sarcoplasmic reticulum is probably the intracellular Ca pool involved with Ca release for contraction, it is possible that diminished contractility in failing hearts may be related to impairment of the sarcoplasmic reticulum to bind or release Ca due to lack of high energy phosphates stores or to a direct effect of hypoxia on the sarcoplasmic reticulum. As the sarcoplasmic reticulum becomes depleted of Ca, there would be less Ca available for release to initiate contraction.

Various laboratories have studied the Ca binding and releasing capacity of sarcoplasmic reticulum obtained from various mammalian failing hearts and have reported depressed capacity or rate of Ca binding, uptake and release from these hearts. The rate of Ca uptake was found to be depressed in sarcoplasmic reticulum fragments prepared from hearts of spontaneously failing canine heart-lung preparations (Gertz et al., 1967), from canine hearts ischemic for 60 or 90 min (Lee et al., 1967), from right ventricles of calves failing from experimentally induced pulmonary

hypertension (Suko et al., 1970), from rabbit hearts failing from experimentally produced aortic insufficiency (Ito et al., 1974) and from failing cardiomyopathic Syrian hamster hearts (Gertz et al., 1970; Sulakhe and Dhalla, 1971). Ca binding capacity of microsomes and mitochondria of isolated rat hearts perfused with substrate-free media has also been found to be depressed (Muir et al., 1970). This reduction of Ca binding by the sarcoplasmic reticulum and the mitochondria was accompanied by a decline of myocardial contractility. The rate of Ca uptake and release from reticulum fragments of failing human hearts, made available at the time of transplantation was reported to be lower than non-failing human hearts (Schwartz et al., 1972) and a variety of normal animal hearts (Harigaya and Schwartz, 1969). The rate of Ca binding (McCollum et al., 1970), uptake and release by cardiac sarcoplasmic reticulum of failing cardiomyopathic Syrian hamster have also been found to be impaired (Schwartz, 1972). Schwartz et al., (1973) further demonstrated that rate of Ca release by the sarcoplasmic reticulum was impaired 12 min after the onset of ischemia. At this time there was no change in Ca binding or releasing capacity of other membranous organelles. Schwartz therefore suggested that the impairment of Ca release from the sarcoplasmic reticulum was the cause of poor contractility associated with early ischemia.

It is well known that in hypoxia or ischemia, the oxidative metabolism of glucose is converted to anaerobic glycolysis (Cornblath et al., 1963; Williamson, 1966; Jennings, 1970 and Opie, 1971/72), which results in accumulation of lactic acid. This end product of glycolysis then decreases intracellular pH. Katz and Hecht (1969) and Katz (1971/72, 1973)

observed that the affinity of troponin for Ca was decreased in acidosis and proposed a competitive interaction between H and Ca for binding sites on troponin A in the ischemic heart muscle. Thus, an intracellular metabolic acidosis could cause a reduction of Ca binding to troponin in myocardial ischemia which would then result in a depression of contractility.

It is also known that the affinity of the sarcoplasmic reticulum for Ca depends on pH (Hasselbach et al., 1969, Sreter, 1969). Nakamaru and Schwartz (1970) reported that when the pH was abruptly increased from 6.7 to 7.8, isolated cardiac sarcoplasmic reticulum lost its affinity for Ca even in the presence of ATP. Alternately, decreasing the pH from 6.7 to 5.9 caused an increase of Ca binding to cardiac sarcoplasmic reticulum. The results indicated that in myocardial hypoxia or ischemia, the intracellular acidosis resulted from the accumulation of lactate may interfere with the release of Ca by the sarcoplasmic reticulum, thus causing an impairment of contractility.

Another possible cause for the immediate reduction of contractility in myocardial ischemia could be due to a reduction in membrane-bound Ca that can be released during excitation to activate contraction either directly, or indirectly by displacing additional Ca from other intracellular depots. Indeed, Nayler and Merrillees (1971) and Nayler et al., (1971) reported a reduction in the amount of Ca that could be displaced by La from superficially-located membrane binding sites after 10 min of hypoxia or 5 min of ischemia when contractility was depressed. She was unable to detect alterations of Ca binding or uptake of cardiac microsomes at this time.

C. Ca Induced Myocardial Necrosis.

a. Ischemia and hypoxia induced necrosis.

It has been shown that myocardial cells subjected to transient ischemic injury markedly accumulate Ca (Shen and Jennings, 1972a). Much of this increased Ca was found in mitochondria (Shen and Jennings, 1972a). There is little doubt that mitochondria can act as sinks for Ca in the heart (Lehninger, 1974; Carafoli and Azzi, 1972). Heart mitochondria are known to have the ability to accumulate excess Ca that has entered the cell (Legato et al., 1968; Carafoli, 1975). Indeed, the accumulation of Ca in mitochondria of failing hearts has been reported by different investigators. Schwartz (1971/72) reported increased Ca accumulation in mitochondria of hypertrophic and failing cardiomyopathic Syrian hamster hearts. Ito and Chidsey (1972) reported an accumulation of Ca in mitochondria of rabbit hearts failing from experimental aortic insufficiency. There was, however, no change in total tissue Ca. Kim and Harrison (1974) reported reduced contractility and increased Ca binding in mitochondria of chronic K-deficient hearts. Shen and Jennings (1972a) studied the effect of ischemia on myocardial electrolyte levels. They reported marked elevation of mitochondrial Ca in dog myocardial cells subjected to 40 min of ischemia followed by 20 min of reperfusion. There was, however, no accumulation of mitochondrial Ca in cells subjected to 60 min of ischemia or 10 min of ischemia followed by 20 min of arterial reflow (Shen and Jennings, 1972b). Thus, the accumulation of mitochondrial Ca depends on the duration of ischemia as well as arterial reperfusion. Investigation of the localization of the increased Ca by cellular fractionation followed by spectrophotometry as well as by electron microscopy and

microincineration showed that much of the Ca was localized in dense bodies within the mitochondria. Jennings and Ganote (1974) observed that mitochondria in both severely ischemic and irreversibly damaged cells became swollen, developed enlarged matrix with small amorphous matrix densities, had disorganized cristae, and showed marked functional defects.

The presence of electron-dense deposits has also been observed in isolated liver (Greenawalt et al., 1964; Hackenbrock and Caplan, 1969) and heart (Brierley et al., 1963; Brierley, 1964) mitochondria incubated in a medium containing Ca and phosphate ions. Brierley et al., (1963) reported that this uptake of Ca and phosphate into the matrix is an energy-dependent process. Hackenbrock and Caplan (1969) reported that the Ca uptake resulted in swelling and transformation of the mitochondria from a condensed to an orthodox conformation which is different from mechanochemically induced orthodox conformation in that its development is associated with a marked decrease in acceptor control and oxidative phosphorylation efficiency. Therefore accumulation of Ca by the mitochondria may lead to a reduction of ATP production and cell damage.

b. Isoproterenol-induced necrosis.

Intraperitoneal administration (I.P.) of a large dose (40 mg/kg) of isoproterenol to rats has also been reported to result in increased mitochondrial Ca accumulation (Nirdlinger and Bramante, 1974). The mitochondria examined 24 hours after the administration of the drug showed signs of structural damage, changed configuration from condensed to orthodox ultrastructure, became swollen or ruptured with cristae in various stages of disruption. Fleckenstein (1970) and Fleckenstein et al., (1974) reported increased Ca accumulation in hearts of

rats given a high I.P. dose (30 mg/Kg) of isoproterenol. Together with the myocardial Ca overload, there was loss of 50% of ATP and 85% creatine phosphate in the myocardium and development of severe disseminated myocardial necrosis. Fleckenstein suggested that increased binding of Ca caused the mitochondria to swell and to deteriorate in respiratory and phosphorylating capacity. Deficient ATP synthesis together with increased ATP breakdown caused by β-adrenergic overstimulation of the heart resulted in a deleterious reduction in high energy phosphate levels. Thus cardiac function and structural integrity of the cell could not be maintained in animals receiving large doses of isoproterenol. Bloom and Cancilla (1969) observed cellular necrosis together with mitochondrial calcification in hearts of rats administered isoproterenol by intraperitoneal injection at a dose of 0.5 to 5 mg/Kg. Mitochondria examined 8 min after drug administration showed deposits of electron-dense material similar to those seen in myocardial ischemic injury (Shen and Jennings, 1972a, 1972b; Jennings and Ganote, 1974) and in cardiac necrosis in experimental Mg deficiency (Heggtveit et al., 1964; Heggtveit and NadKarni, 1971). However, the mitochondria appeared normal as shown by electron microscopy. This suggests that the mitochondria have sufficiently normal electron transport and oxidative phosphorylating capacity to allow active uptake and storage of Ca within 8 min of isoproterenol administration. There was increase of myocardial Ca accumulation, as the dose of isoproterenol was increased from 10^{-4} to 10^{-1} mg/Kg (Bloom and Davies, 1974) but no further increase of Ca uptake occurred with doses of isoproterenol larger than 10^{-1} mg/Kg. In contrast, very little myocardial necrosis occurred

at 5 x 10^{-1} and 1 mg/Kg of isoproterenol. However, the amount of necrosis increased in proportion to the dose of isoproterenol used up to approximately 85 mg/Kg (Rona et al., 1959; Chappel et al., 1959). If increased mitochondria Ca binding is responsible for cellular necrosis as Fleckenstein et al., (1974) have suggested, then it remains to be explained why the myocardial necrosis which resulted from doses of isoproterenol larger than 10^{-1} mg/Kg of isoproterenol was greater when no further Ca accumulation has occurred with these larger doses.

Although many investigators reported an increased mitochondrial Ca accumulation in failing hearts, others have reported reduced Ca accumulation in these hearts. Mitochondria from failing cardiomyopathic hamster hearts have been reported to have depressed ability to accumulate Ca (Schwartz et al., 1968; Lindemayer et al., 1970; Sulakhe and Dhalla, 1971). The mitochondria isolated from these failing hearts also have depressed respiratory control (Schwartz et al., 1968; Lindemyer et al., 1970). Tomlinson and Dhalla (1973) observed reduced mitochondrial Ca content in isolated rat hearts perfused with a substrate-free medium for 2 hours, with a hypoxic medium for 30 min or with substrate-free hypoxic medium for 10 min.

D. The Effect of β-blockers on Contractility and Ca Metabolism.

The use of propranolol, a β-adrenergic blocking agent for angina pectoris, is well-documented (Alleyne et al., 1963; Keelan, 1965; Aronow, 1972). Propranolol has also been reported to be effective in limiting the extent of myocardial damage subsequent to coronary occlusion (Maroko et al., 1969, 1971, 1972, 1973; Khan et al., 1972; Sommers and Jennings, 1972; Reimer et al., 1973) and hypoxia (Wildenthal et al., 1973). The

protective effect of propranolol may be due to myocardial depression (Nayler et al., 1969; Maroko et al., 1971, 1973) thus reducing the oxygen requirement of the heart and/or redistribution of blood flow and preventing the occurrence of relative endocardial ischemia (Becker et al., 1971). Jennings and Reimer (1974), however, observed no redistribution of blood flow in the ischemic myocardium after the administration of propranolol. In addition, nonspecific myocardial depression produced by propranolol may be harmful to the already depressed myocardium since further myocardial depression may further reduce perfusion of ischemic area (Stephen, 1966; Aronow, 1972; Gomoll and Braunwald, 1973). On the other hand, practolol, a specific β_1-adrenergic blocking agent (Dunlop and Shanks, 1968; Wasserman and Levy, 1974; Lertora et al., 1975) has also been reported to reduce the extent of myocardial ischemic injury in vivo (Libby et al., 1973; Khan et al., 1972; Pelides et al., 1972; Lekven, 1975) but without appreciable depression of myocardial function (Dunlop and Shanks, 1968; Matlof et al., 1973; Thompson et al., 1974). It is thus possible that at least a part of the protective effect of practolol is not related to reduction of oxygen requirements. Perhaps inhibition of myocardial Ca accumulation in hypoxia or ischemia is responsible for the protective effect of practolol.

The ability of propranolol to inhibit microsomal Ca uptake (Hess et al., 1968; Shinebourne et al., 1969; Katz et al., 1974) and binding (Pretorius et al., 1969; Katz et al., 1974) has been reported. Hess et al., (1968) and White and Shineborune (1969) believed that this inhibition of Ca uptake is mediated by a β-adrenergic blocking action of

propranolol since the inhibition is reversed by β-adrenergic agonists. Furthermore, propranolol prevented the epinephrine induced cardiac microsomal Ca uptake (Entman et al., 1969). In contrast, Scales and McIntosh (1968), Watanabe and Besch (1974) and Katz et al., (1974) reported that l-propranolol and d-propranolol, which lacks β-blocking action, were both effective in inhibiting microsomal Ca uptake. Also, this inhibition of Ca uptake and Ca binding was not reversed by isoproterenol (Katz et al., 1974).

A much lower dose of propranolol than that which inhibit microsomal Ca uptake was found to be effective in blocking catecholamine induced Ca-dependent action potentials together with elevation of cyclic adenosine monophosphate (cAMP) in K-depolarized hearts (Watanabe and Besch, 1974). This drug was unable to block Ca-dependent action potentials induced by high external Ca concentration. It is thus obvious that propranolol did not block Ca-dependent action potentials by acting directly on slow Ca channels as mediated by Ca-antagonists such as verapamil and D600 (Watanabe and Besch, 1974). Rather, the drug indirectly blocked slow Ca-channels by blockade of β-adrenergic receptors. It is thus interesting to find out if practolol, which has also been found to be effective in reducing the size of a myocardial infarct, has any effect on myocardial Ca exchange.

II. Statement of Problem.

A. <u>The Effect of Hypoxia on Excitation-contraction Coupling.</u>

Different mechanisms have been proposed to account for the early reduction of contractile activity associated with ischemia or hypoxia. It now appears that early contractile failure may be associated with a

decrease in the availability of Ca involved in coupling excitation to contraction. The question remains as to whether the reduction of contractile activity was a result of a decreased Ca release from the sarcoplasmic reticulum or superficially-located membrane binding sites or both. The first objective of my study was to determine if the reduction of contractility in early hypoxia is associated with alterations of Ca exchange by different Ca pools in the hearts: a small, superficial and relatively labile Ca pool Ca_1 which may modulate the release of Ca from a larger Ca pool, Ca_{II} or Ca_2 (Bailey and Ong, 1974) which has been shown to be directly related to the maintenance of contractile force in the heart (Bailey and Dresel, 1968). The hypothesis to be tested is that the early reduction in contractile activity is a consequence of distortions in the Ca content of one or more of the pools involved in excitation-contraction coupling.

B. The Effect of Isoproterenol on Excitation-contraction Coupling and Ca Metabolism

Hypoxia and ischemia, like the administration of isoproterenol have been reported to cause myocardial necrosis. The damage caused by these insults has been suggested to be a result of increased Ca accumulation particularly in the mitochondria. However, it is well known that hypoxia or ischemia reduces myocardial contractility possibly by reducing the availability of Ca for contraction while on the other hand, the administration of isoproterenol increases contractility as a result of an increased transmembrane Ca influx. Thus there would appear to be a discrepancy between the effects of hypoxia and isoproterenol on excitation-

contraction coupling and their effects on cellular integrity. Therefore, the second objective of this study was to compare the effect of isoproterenol to that of hypoxia on excitation-contraction coupling and the acute effects of both treatments on Ca metabolism. The hypothesis to be tested is that both treatments have similar effects on total tissue Ca content and especially mitochondrial Ca content but dissimilar effects on the Ca involved in excitation-contraction coupling.

C. The Effect of Practolol on Changes of Excitation-contraction Coupling and Ca Metabolism Produced by Hypoxia or Isoproterenol.

The protective effect of practolol, a β_1 adrenergic blocking agent, on the extent of myocardial ischemic injury has been well-documented. Since both ischemia and isoproterenol are believed to induce the development of cellular necrosis by the same mechanism, that is, an overload of Ca in the mitochondria, it is logical to assume that practolol may protect against the changes in Ca metabolism and perhaps changes in the Ca pools involved in excitation-contraction coupling caused by ischemia, hypoxia or the administration of isoproterenol. The final objective of this study was to determine the effect of practolol on the distortions of Ca exchange induced by hypoxia and treatment with isoproterenol. The hypothesis to be tested is that practolol prevents the phenomenon of "Ca overload" in the acute stage of hypoxia and isoproterenol insult by preventing the accumulation of abnormal quantities of total tissue Ca. An ancillary objective to the study is that practolol may prevent or ameliorate the reduction in contractility seen with hypoxia or the increase in contractility seen with isoproterenol by modifying the distortions in Ca content of the pools associated with excitation-contraction coupling

caused by these treatments.

III. Approach to Problem.

To study alterations of Ca exchange that are associated with contractile failure in early ischemic or hypoxic injury, an isolated, hypoxic heart preparation was used to mimic the condition of an ischemic myocardium. Hypoxia was produced by perfusing the isolated mammalian heart with a medium equilibrated with little or no oxygen and containing no erythrocytes (Weissler et al., 1968). Acute coronary occlusion was not used in this investigation to study Ca exchange in ischemic myocardium since this method is associated with difficulties in sampling perfusate from an ischemic area due to reduced flow and mixing of perfusate coming from ischemic and non-ischemic areas of the heart. In an isolated hypoxic heart preparation it is possible to produce any degree of hypoxia by using different concentrations of oxygen to equilibrate the perfusion medium. The generalized hypoxic myocardium produced this way is different from the clinical picture of myocardial ischemia in that the arterial in-flow is maintained to continuously supply substrates to and remove metabolites from the heart. Nonetheless, perfusion studies allow more precise definition of derangements of the myocardium under insufficient oxygen supply.

Hypoxia was produced in isolated kitten hearts by perfusing the hearts with a modified Krebs-Henseleit (K-H) solution equilibrated with 5% O_2, 5% CO_2 and 90% N_2. The hypoxic medium with an oxygen tension (pO_2) of approximately 40 mm Hg causes an immediate reduction of myocardial contractility. After 15 min hypoxic perfusion, contractility can be re-

covered to the original level by perfusion of hearts with a normoxic medium (pO_2= 600 mm Hg). Thus, it is possible to use the same hearts for control and treatment. To eliminate changes in Ca metabolism caused by time, a cross-over experimental design was used so that the order of control and treatment was reversed in half of the hearts studied.

Ca exchange was studied under nonsteady-state conditions so that changes in contractility can be related to changes in Ca fluxes (Bailey and Ong, 1974). Thus, by perfusion with a Ca-free K-H solution, it was possible to relate the washout of a single Ca compartment to the decay of contractile force (Bailey and Dresel, 1968). Moreover, by reperfusion of Ca-depleted hearts with a Ca-containing K-H solution, it was possible to relate the restoration of contractile force to the uptake of Ca (Bailey and Sure, 1971).

To study the effect of isoproterenol on excitation-contraction coupling, a dose of isoproterenol to produce a 70-100% increase of contractility was selected. The dose of drug required was determined in each experiment prior to study of Ca-exchange under nonsteady-state conditions. To study the deleterious effect of isoproterenol on Ca metabolism, a dose of isoproterenol which caused a 100-200% increase of contractility was selected. The drug was infused for 25 min prior to Ca-free washout and uptake.

Practolol at 10^{-5}M was used to study its effect on hypoxia or isoproterenol induced changes of contractility and Ca metabolism. It was reasoned that this dose of practolol which blocked the positive inotropic effect of 10^{-7}M of isoproterenol would probably modify the isoproterenol or hypoxia-induced distortions of Ca involved with coupling excitation to contraction.

SECTION II.

METHODS

I. Experimental Preparation.

A. The perfusion apparatus.

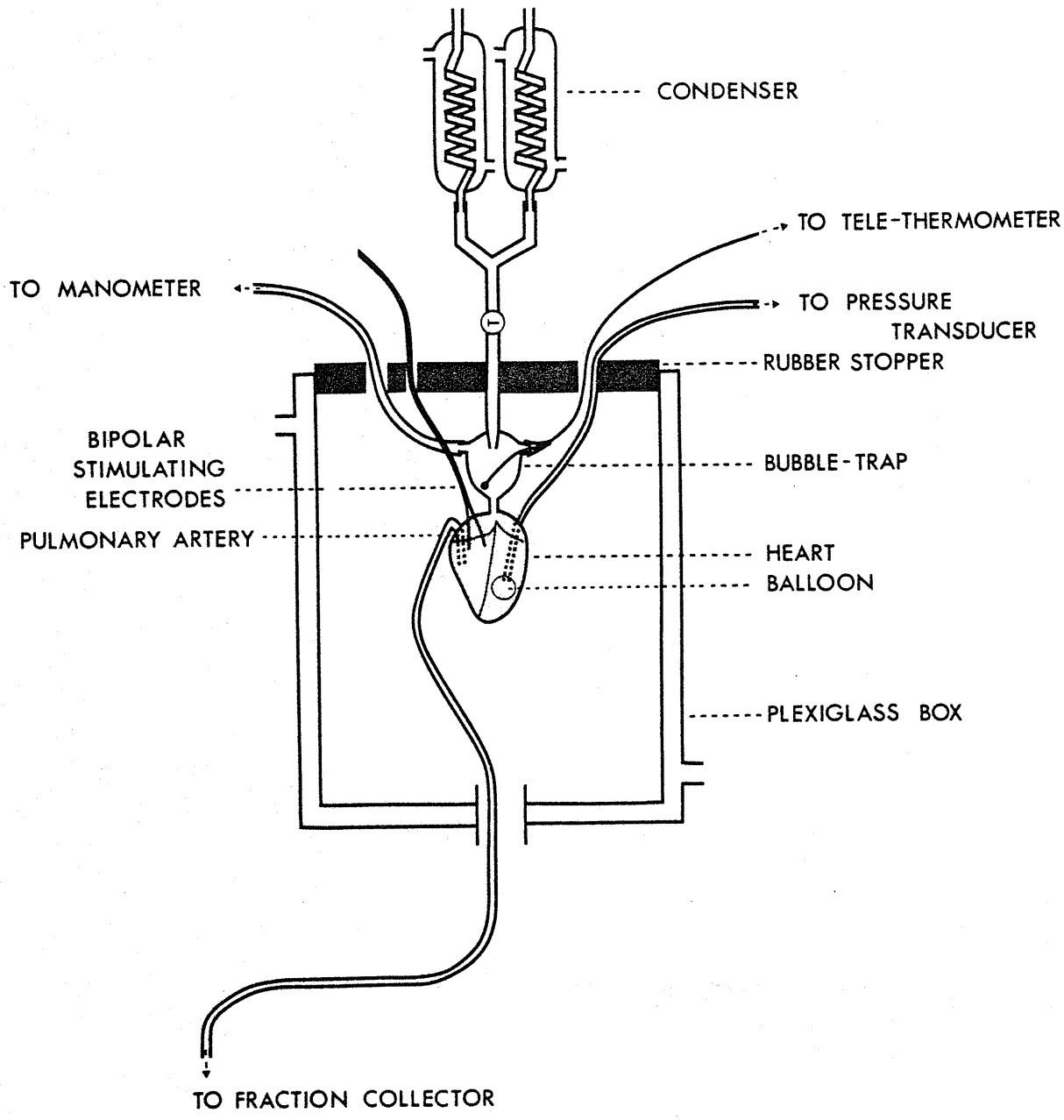
A schematic diagram of the perfusion apparatus used in this study is shown in Figure 1. The hearts were perfused by the Langendorff technique. The hearts were attached to a bubble-trap connected through a three-way stopcock to two spiral condensers for warming the liquid perfusates. A thermistor probe (YSI Model 403) was inserted through one side arm of the bubble-trap and connected to a tele-thermometer (YSI Model 43TA) for measurement of perfusate temperature. Perfusate temperature was maintained by means of a Heto Thermostat (Birkerød, Denmark, Model 623) at 37.0 $\pm$ 0.5°C. The other side arm of the bubble-trap was connected to a pressure transducer (Statham P23B) for measurement of perfusion pressure. Perfusion pressure was monitored by an aneroid manometer (Industrial and Scientific Instrument Co., Philadelphia, U.S.A., Model 1331) and maintained at 70 mm Hg throughout the experiment by alteration of the perfusate flow. A Masterflex Pump (Cole-Palmer Model 7598), was used to deliver perfusate to the preparation, and was monitored by a Masterflex Tachometer (Cole-Palmer Model 7598) calibrated prior to the start of the experiment to read flow in ml/min.

The perfused heart, after being attached to the perfusion apparatus was maintained at a temperature of 37.0 $\pm$ 0.5°C in a water-saturated environment in an enclosed water-jacketed Plexiglass chamber.

B. Preparation of the heart.

Kittens of either sex weighing 0.6 - 1.2 kg were injected with heparin (1000 µ/kg) (Connaught Labs, Toronto, Canada) intramuscularly one hour before being killed by a blow to the head. The hearts were immediately removed and placed in cold (4°C) modified Krebs-Henseleit solution

Figure 1: The perfusion apparatus (see text for description).


CONDENSER
TO TELE-THERMOMETER
TO MANOMETER
TO PRESSURE TRANSDUCER
RUBBER STOPPER
BIPOLAR STIMULATING ELECTRODES
BUBBLE-TRAP
PULMONARY ARTERY
HEART
BALLOON
PLEXIGLASS BOX
TO FRACTION COLLECTOR

(see below). Extraneous tissue was removed and the heart was attached by the aorta to the bottom of the bubble-trap as shown in Figure 1. An incision was made in the pulmonary artery and a cannula was introduced into the right ventricle for collection of effluent from the heart. The effluent was led out of the Plexiglass chamber through the hole at the bottom of the chamber. To prevent loss of perfusate from the right atrium, the venae cavae were ligated. A small incision was made in the apex of the left ventricle to allow escape of perfusate which had leaked through the aortic valve and the effluent from minor deep myocardial veins (Thebesian and sinusoidal channels) which empties into the left ventricle. An incision was made in the auricular appendage of the left atrium and a saline-filled balloon was inserted through the mitral valve into the left ventricle and inflated to produce an end diastolic pressure of approximately 5 mm Hg. The hearts were required to contract against this load and were thus performing isometric work. Intraventricular pressure and its first derivative (dP/dt) were recorded on a Brush 220 recorder and were used as indices of contractility. The hearts were driven electrically at 180 beats/min by a Grass SD5 Stimulator at twice threshold voltage through bipolar electrodes affixed to the right atrium.

II. Composition of Perfusion Solutions

The liquid perfusate used to equilibrate the hearts was modified Krebs-Henseleit (1932) solution (K-H solution) equilibrated with 95% O_2 and 5% CO_2 to give a pH and pO_2 of approximately 7.4 and 600 mm Hg, respectively. The modified K-H solution had the following composition:

	mM	%
NaCl	112.2	
KCl	4.5	
KH_2PO_4	1.2	
$NaHCO_3$	26.2	
$MgSO_4$	1.2	
$CaCl_2$	1.8	
Glucose	11.1	
Dextran 40 (Abbot Labs)		1.0

The calculated osmolarity of the above solution was between 320 and 330 mosm. All perfusion solutions were prepared fresh on the day of the experiment from five stock solutions which contained the following:

		mM	%
1.	NaCl	114.1	
	KCl	5.2	
2.	KH_2PO_4	654.7	
	$NaHCO_3$	29.3	
3.	$MgSO_4$	235.3	
4.	$CaCl_2$	360.2	
5.	Dextran 40		10

The five solutions were stored separately at $4^{o}C$ until use. The KH_2PO_4 + $NaHCO_3$ solution (solution 2) was bubbled with 100% CO_2 for 1 hour as described by Krebs and Henseleit (1932).

To prepare 1 litre of K-H solution, 95 ml of Dextran 40 was added to 855 ml of solution 1. The resultant solution was stirred with a magnetic stirrer and bubbled with a normoxic or hypoxic gas mixture for

30 min. Forty ml of solution 2, 5 ml of solution 3, 5 ml of solution 4 and 2 g of glucose were added to the mixture of solutions 1 and 5 with continuous stirring and bubbling.

In hypoxic perfusions, the hypoxic K-H solution was equilibrated with 5% O_2, 5% CO_2 and 90% N_2 to give a pH and pO_2 of approximately 7.4 and 40 mm Hg, respectively.

Ca-free K-H solutions differed only in that $CaCl_2$ was omitted from the K-H solution.

Practolol (Ayerst Labs, Montreal, Canada) was added to the K-H solution to give a final drug concentration of 1×10^{-5}M. This concentration of practolol completely blocked the inotropic response to 10^{-7}M of l-isoproterenol (Sigma Chemical Corp., St. Louis, U.S.A.).

L-isoproterenol was prepared fresh daily by diluting an aliquot of a stock solution 100 to 200 fold with saline and was kept at 4°C until used to prevent oxidation of the drug. The stock solution contained 10^{-3} g/ml of l-isoproterenol in saline to which 2×10^{-4} g/ml of ascorbic acid was added as an antioxidant. It was kept frozen until needed.

III. Experimental Procedures.

A. Perfusion protocol.

a. Normoxic perfusion

After the hearts were attached to the perfusion apparatus, they were perfused with a normoxic K-H solution until contractile activity had reached a steady state, usually within 30 min. The hearts were then perfused with a Ca-free normoxic K-H solution after a brief period of gas perfusion with a normoxic gas mixture (95% O_2 and 5% CO_2) to prevent intermixing of Ca-containing and Ca-free perfusates. Ca-free perfusion was continued until systolic intraventricular pressure was reduced to less than 5 mm Hg (Wash I). This took approximately 3 min. After a

brief gas-perfusion to prevent intermixing of liquids, the hearts were perfused for 3 min with a Ca-containing normoxic K-H solution to which trace amount of ^{45}Ca (New England Nuclear, Quebec, Canada) was added (Ca-uptake). The hearts were again perfused for approximately 3 min with a normoxic Ca-free K-H solution (Wash II) after another brief period of gas perfusion. After Wash II, the hearts were allowed to recover by perfusion with the normoxic K-H solution for more than 30 min.

b. Hypoxic perfusion

To provide an internal control, each heart was used for both the normoxic as well as the hypoxic perfusion. That is, a group of hearts were first perfused with normoxic Ca-containing as well as Ca-free solutions as described. After Wash II and at least 30 min of recovery, these hearts were perfused with a hypoxic K-H solution for 3 min. They were then subjected to approximately 3 min each of hypoxic Wash I, Ca-uptake and Wash II using a hypoxic gas mixture (5% O_2, 5% CO_2 and 90% N_2) for separation of Ca-free and Ca-containing perfusates. The perfusion protocol was reversed in another group of hearts to provide a cross-over experimental design. That is, the hearts were first subjected to hypoxic and then normoxic perfusion of the same time duration as above. It should be pointed out that after approximately 13 min of total hypoxic perfusion, dP/dt could be restored to normal levels upon 30 min of reoxygenation, thus allowing assessment of Ca exchange kinetics in the same heart for control and hypoxic treatment.

At the end of the experiment, the hearts were briefly gas-perfused to clear the coronary vasculature of liquid. The hearts were then removed from the perfusion apparatus, blotted dry on filter paper, weighed

and ashed overnight for the determination of residual tissue Ca.

c. Practolol perfusion

In another group of hearts, the effect of practolol on normoxic and hypoxic perfusion was studied. After 30 min of equilibration, the hearts were perfused for 3 min with practolol K-H solution prior to Ca-free washout (Wash I), Ca-uptake and another Ca-free washout (Wash II). Perfusion with practolol was continued during Wash I, Ca-uptake and Wash II. Afterwards, contractility was allowed to recover in the absence of practolol. Again, a cross-over design was used so that a group of hearts was subjected first to normoxic and then hypoxic perfusions in the presence of practolol, and another group of hearts was perfused first with hypoxic and then normoxic practolol K-H solutions. At the end of the experiments, the hearts were briefly gas-perfused, dried, weighed and ashed for the determination of residual tissue Ca.

The effect of practolol on restoration of contractility after exposure to 3 or 10 min of hypoxic perfusion was also determined. The hearts were equilibrated for 30 min prior to 3 min of perfusion with a hypoxic K-H solution. They were then perfused with a normoxic K-H solution to determine the rate of restoration of contractility (dP/dt) in the absence of practolol. Thirty min of reoxygenation was allowed for the recovery of dP/dt after hypoxic perfusion in all cases. However, the rate of restoration of dP/dt was followed for 10 min. After recovery, the hearts were perfused for 3 min with a practolol K-H solution prior to 3 min perfusion with a hypoxic practolol K-H solution. The hearts were then allowed to recover for 10 min with a normoxic

practolol K-H solution in order to compare the rate of restoration of dP/dt after 3 min of hypoxia in the absence to the presence of practolol.

The effect of practolol on the rate of restoration of dP/dt after 10 min of hypoxia was also determined in the same hearts after another 20 min of perfusion with a normoxic K-H solution in the absence of practolol. The hearts were perfused for 3 min with a practolol K-H solution prior to 10 min of perfusion with a hypoxic practolol K-H solution. The hearts were allowed to recover by 10 min perfusion with a normoxic practolol K-H solution. This was followed by 20 min of normoxic perfusion in the absence of practolol. Finally, the hearts were perfused for 10 min with a hypoxic K-H solution followed by 10 min of perfusion with a normoxic K-H solution in the absence of practolol. Control measurements of dP/dt were taken immediately prior to the hypoxic insult. Subsequent changes in contractility were assessed at 0.5 to 2 min intervals during hypoxic perfusion and during recovery.

d. Isoproterenol perfusion

Isoproterenol was infused into the perfusion medium with a Harvard Infusion Pump (Model 600-000) at different rates to determine the concentration which produced a 70 - 100% increase of dP/dt. The infusion was then stopped. After the return of dP/dt to control levels, this concentration of isoproterenol was infused during Wash I, Ca-uptake and Wash II. After the hearts had recovered subsequent to Wash II, they were treated with practolol during Wash I and II and during Uptake in the presence of isoproterenol. A cross-over design was used in the study so that another group of hearts were subjected to similar treatments but with the order reversed, that is, the effect of isoproterenol was determined first, in the presence and then, in the absence of practolol.

At the end of the experiment, the hearts were briefly gas-perfused, blotted dry and weighed.

B. Analytical methods.

a. Determination of oxygen tension.

To determine oxygen tension, samples of perfusate and effluent were collected anaerobically in a syringe. The partial pressure of oxygen in these samples was determined under anaerobic conditions on a Radiometer Blood Gas Meter (Radiometer PHM27, Denmark). Oxygen-free solution was prepared by dissolving 100 mg of sodium sulphite crystals in 5 ml of 0.01 M borax solution inside a closely capped syringe. Calibration was accomplished with three solutions equilibrated for 30 min with different proportions of oxygen, namely, 5% O_2, air and 95% O_2 .

b. Determination of ion concentrations.

The effluent from the hearts during the Ca-free washouts and Ca-uptakes was collected at 6 sec intervals with a fraction collector (ISCO, Model 272) via a cannula inserted into the right ventricle through the pulmonary artery. The volume of each sample was measured. A 0.5 ml aliquot of each sample from the Ca-free washout and a 0.2 ml aliquot from Ca-uptake was diluted with 2 ml of a 1% La solution in 5% HCl. The Ca concentration in these samples was determined by atomic absorption spectrophotometry at 422.7 mμ (Perkin-Elmer, Model 303). For each set of ion determinations, a standard curve was prepared by dissolving a series of different concentrations of Ca in the La solution (0, 0.05, 0.10, 0.25, 0.40, 0.50 mEq/L).

The ^{45}Ca concentration in samples collected during Ca-uptake and

Wash II was determined by liquid scintillation counting (Liquid Scintillation Analyser, Phillips, Model PW4510). A 10 ml scintillation fluid was added to 50 µl aliquot of each sample. Quench corrections for ^{45}Ca activity were found to be unnecessary since counting efficiency did not vary significantly from sample to sample. The scintillation fluid had the following composition:

PPO (2,5-diphenyloxazole; Calbiochem.)	11.3	g
POPOP (1, 4-bis-2-[5-phenyloxazolyl]-benzene; Calbiochem.)	0.2	g
Toluene (Fisher Scientific)	2	l
Ethylene glycol monomethyl ether (Fisher Scientific)	500	ml

The primary fluor, PPO and secondary fluor, POPOP were first added to toluene, the solvent, with continuous stirring. After dissolution of the solutes, ethylene glycol monomethyl ether was added to the resultant solution to allow accomodation of small amounts of aqueous samples for counting.

At the conclusion of the experiments the hearts were removed from the perfusion apparatus, atrial tissue was removed, the ventricles were blotted on filter paper and weighed. Approximately 1 g of ventricular tissue was excised from each heart. This sample was blotted dry on filter paper, weighed in a platinum crucible, dried overnight and then ashed for 8 hours at 600°C in a Thermolyne muffle furnace (Thermolyne Corp., Type 2000). Temperature was increased in stages to 600°C to prevent spattering of sample. Two mls of concentrated HCl was added to the residue to dissolve the inorganic ions. A 0.5 ml aliquot of this sample was added to 1 ml of the 1% La solution in 5% HCl. Ca concentration in this sample was determined by atomic absorption spectro-

photometry. The amount of Ca in the muscle was expressed as mEq/kg wet wt.

c. Graphical analysis of Ca-washout and uptake data

The logarithm of Ca concentrations measured in the effluent samples during Ca-free washouts was plotted as a function of time. The resultant curve obtained was that of a typical three compartment washout curve (Figure 2). Compartmental analysis of the Ca washout data was done as outlined by Riggs (1963) and Bailey and Ong (1973). The usual manual technique was modified by a computerized technique (Vivian & Bailey, in preparation) which did not require the number of compartments to be specified or the kinetic parameters to be estimated prior to analysis. Decisions regarding the number of compartments and the parameters describing each compartment were made only on the basis of statistical criteria. Therefore, use of this technique minimized the extent of personal bias inherent in the usual graphical compartmental analysis. Graphical analyses of Ca washout curves by the usual "curve peeling" procedure of randomly selected data did not yield results for kinetic analyses differing significantly from the computerized statistical procedure. Briefly, computerized compartmental analysis was done on a DEC PDP8/e computer (Digital Equipment Corp.) as follows: The least squares best fit line for the last four data points from a washout curve was calculated and became a "tentative compartment". Subsequent data points were tested. If four of the subsequent data points (P1, P2, P3, P4) were above the best fit line and if both the third (P3), and fourth (P4) points deviated from the line by more than one standard deviation, another best fit line was calculated for the four data points. The

slope of this line was compared with the slope of the "tentative compartment". If the two slopes did not differ significantly ($P < 0.05$), then P1 was included into the current tentative compartment and the four boundary test points were moved one point to the left. Each subsequent data point was tested in the same way. If, on the other hand, the slopes were significantly different ($p<0.05$), then the tentative compartment became permanent, and the second best fit line became a second "tentative compartment". To evaluate the "tentative comparment", the data were reduced by subtracting or peeling the now permanent compartment from each of the washout data points and subsequent data points were tested as described above until the washout curve was analyzed. The equation yielded by kinetic analysis to describe the washout of Ca was as follows:

$$[Ca]_t = [Ca_I]_o e^{-k_1 t} + [Ca_{II}]_o e^{-k_2 t} + [Ca_{III}]_o e^{-k_3 t} \qquad (1)$$

The symbols are defined in Table I. The halftime, $T_{1/2}$, for the washout of Ca from each compartment was obtained from the computerized analysis and is related to the slope k_i, by the following relationship:

$$T_{1/2} = 0.693/k_i \qquad (2)$$

The quantity of Ca contained in each compartment was calculated as follows:

$$Ca_i \text{ content} = \frac{\sum_{t=0}^{N} [Ca_i]_t \cdot V_t}{\text{wet wt}} \qquad (3)$$

To analyze the uptake data, the difference between the perfusate Ca concentration, $[Ca]_A$, and the concentration of Ca measured in the effluent from the hearts, $[Ca]_t$, was first obtained. The resultant data plotted logarithmically as a function of time yielded a curve

TABLE I

Definition of Symbols

Symbols	Definition
$[Ca]_t$	Ca concentration (mEq/l) in the effluent at any given time t.
$[Ca_i]_o$	Ca concentration (mEq/l) in the i^{th} compartment of the effluent, where i = I, II or III for washouts and 1 or 2 for uptakes, at time zero.
$[Ca_i]_t$	Concentration of Ca (mEq/l) contained in the i^{th} compartment at any given time t.
$[Ca]_p$	Measured concentration of Ca (mEq/l for ^{40}Ca and cpm/l for ^{45}Ca) in perfusate.
k_i	Rate constant or slope (sec^{-1}) for the washout or uptake of Ca by the i^{th} compartment.
V_t	Volume of effluent (ml) at time t.
Ca_i content	Quantity of Ca (mEq/kg tissue wet wt) contained in or extracted by the i^{th} compartment.
t	Time of collection of sample (sec).
N	Number of samples.

similar to a two-compartment washout curve. Only data points representing samples collected during restoration of dP/dt (and not after dP/dt had reached a steady state) were used for compartmental analysis of Ca uptake. This was required since as Ca uptake approached the steady state, i.e., when $[Ca]_t$ approached $[Ca]_A$, small errors in the measurement of $[Ca_t]$ were magnified out of proportion when converted to logarithms. The omission of data points obtained after the restoration of dP/dt was believed to be valid since the physiological variable in question, dP/dt, had been restored to control levels, and thus subsequent Ca uptake by the heart was not associated with the restoration of contractility and represented only a small fraction of the total Ca uptake. The equation yielded by kinetic analysis to describe the uptake of Ca was as follows:

$$[Ca]_t = [Ca]_A - ([Ca_1]_o e^{-k_1 t} + [Ca_2]_o e^{-k_2 t}) \qquad (4)$$

The resultant difference curve, i.e., $[Ca_1]_o e^{-k_1 t} + [Ca_2]_o e^{-k_2 t}$, was analyzed as a washout curve to yield the intercepts and slopes for the two compartments. Halftimes and intercepts for the uptake of Ca were calculated using the computerized graphical analysis procedure. The quantity of Ca extracted from the heart during the reperfusion by each compartment was calculated by equation (3).

The ^{45}Ca washout and uptake data were analyzed in the same manner as the washout and uptake of ^{40}Ca. To permit comparison of ^{45}Ca contents between experiments and to allow comparison of ^{45}Ca content with ^{40}Ca content within an experiment, all ^{45}Ca contents were adjusted using the perfusate specific activity as follows:

$$\text{Adjusted } {}^{45}Ca_i \text{ content} = \frac{\text{measured } {}^{45}Ca_i \text{ content.} \ {}^{40}[Ca]_p}{{}^{45}[Ca]_p} \qquad (5)$$

where

$$\frac{^{45}[Ca]_p}{^{40}[Ca]_p} = \text{specific activity of } ^{45}Ca$$

d. Graphical analysis of contractile force data

The maximum deflection of the positive derivative of intraventricular pressure, dP/dt, recorded during Ca-free washout was measured at 6 sec intervals. The logarithm of the maximum positive dP/dt was plotted as a function of time.

Compartmental analysis of the decay curve for dP/dt was done as described for the washout of Ca by the computerized technique. In most cases, the decay curve was resolved into two compartments. However, when isoproterenol was used, only one compartment was resolved by the computerized technique. This will be illustrated in the RESULTS section. The half-time for the decay of dP/dt was determined as described for the washout of ^{40}Ca.

C. Subcellular fractionation.

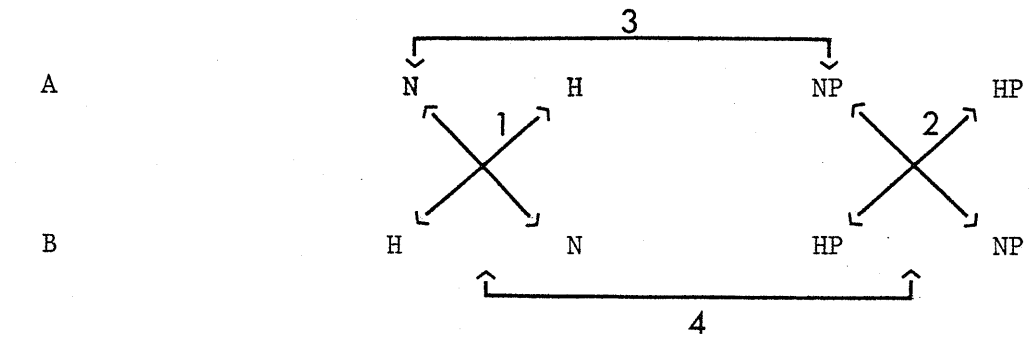
The effect of 10 min of hypoxia on the content of Ca in the mitochondria was determined. Hearts were perfused with normoxic K-H solution for 30 min to obtain a steady level of contractile force. They were then perfused either with a hypoxic K-H solution for 10 min or continued perfusion with the normoxic K-H solution for 10 min. After the perfusion, the hearts were quickly removed from the perfusion apparatus and immersed immediately in cold saline ($4^{\circ}C$). The method described by Sordahl et al. (1970) was used for the determination of mitochondrial Ca content. One to 2 g of the ventricle was minced with scissors and homogenized in 1:12 0.18M KCl and 10 mM EDTA at pH 7.2-7.4 for 3 to 4 sec with a Polytron

Pt-20 tissue homogenizer at a rheostat setting of two. Two quick passes through the homogenate with a motor-driven Teflon pestle were then made to ensure complete cellular disruption. The homogenate was centrifuged at 1,200 x g for 10 min. The supernatant was strained through several layers of cheesecloth and centrifuged at 10,000 x g for 15 min. The resultant mitochondrial pellet was resuspended in the homogenizing medium (approximately half the volume used in the initial homogenization) and centrifuged at 10,000 x g for 8 min. This washing procedure was repeated once to remove cellular contaminants adhering to the mitochondria. The mitochondria were resuspended in the homogenizing medium (approximately 1 ml medium per g of original tissue to yield a suspension containing 2 to 10 mg mitochondria per ml). Protein concentration was determined by the method of Lowry (1951). To prepare the standard curve for the determination of mitochondrial protein, a stock solution containing 5 mg/ml of albumin was prepared. Aliquots of 10, 20, 25, 40 and 50 µl representing dilution factors of 5, 2.5, 2, 1.25 and 1 respectively from the stock solution were pipetted by micropipettes into different cuvettes. Aliquots of 10, 25 and 50 µl of the well-stirred mitochondrial protein suspension were also pipetted into cuvettes. A 0.5 ml 0.1N NaOH was added to all the cuvettes, including the blank in order to dissolve the protein. All solutions were diluted by the method of Lowry (1951) and measured at 620 mµ in a spectrophotometer.

To determine the concentration of Ca in the mitochondrial protein, the protein suspension was stirred by a magnetic stirrer prior to a two to three fold dilution with 1% La solution. Mitochondrial Ca was expressed as mEq Ca/g protein.

IV. Statistical Analysis.

The following diagram illustrates the experimental designs used to evaluate the effects of hypoxia and practolol on the Ca involved in excitation-contraction coupling.



A: groups of hearts subjected to normoxic followed by hypoxic perfusion.

B. groups of hearts subjected to hypoxic followed by normoxic perfusion.

N: normoxic perfusion.

H: hypoxic perfusion.

NP: practolol-treated normoxic hearts.

HP: practolol-treated hypoxic hearts.

Comparison 1 and 2: Cross-over design to evaluate effects of hypoxia in the absence or presence of practolol, respectively.

Comparison 3: Evaluation of the effects of practolol independent of other treatments. Significant time-treatment interaction prevented the use of data from practolol-treated normoxic hearts from second part of Comparison 2 (Group B).

Comparison 4: Test of the effect of practolol on the changes produced by hypoxia.

Cross-over experimental designs were used in the majority of the experiments with the order of control or treatment assigned at random.

Where comparisons were not cross-over, analysis of variance - completely randomized design was used in the statistical analysis. For comparison of experimental means, Duncan's new multiple-range test was used.

SECTION III.

RESULTS

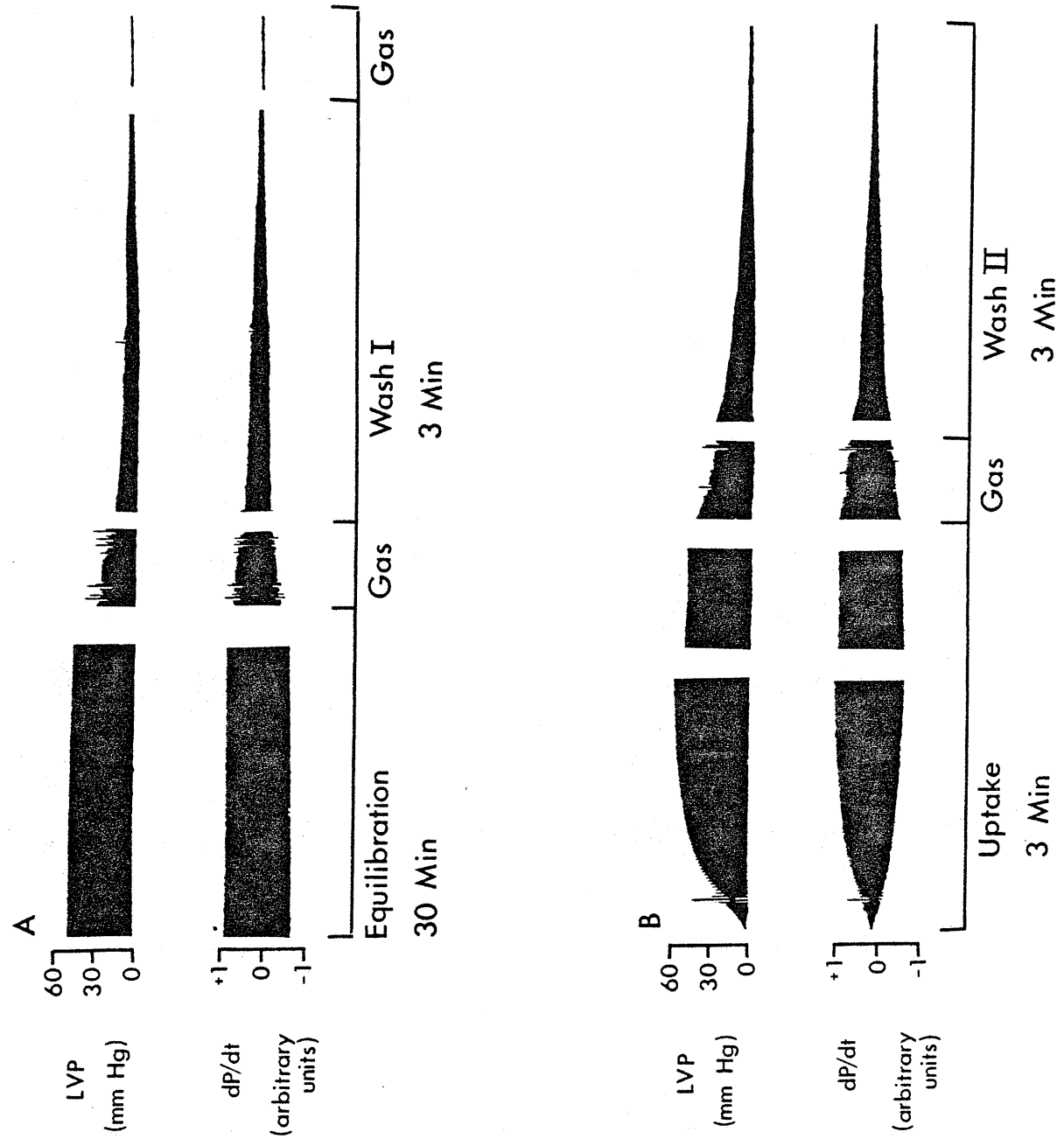
I. Perfusion Protocol.

Figure 2 shows tracings of left intraventricular pressure (LVP) and its time derivative dP/dt, from a typical experiment in which the heart was subjected to Ca-free washouts and Ca-uptake under normoxic conditions. After approximately 30 min of equilibration with the normoxic K-H solution, the heart was gas perfused for approximately 30 sec to clear coronary vessels and perfusion apparatus of perfusate and to prevent mixing of the previous perfusate with that to follow. Perfusion with a Ca-free K-H solution caused the decay of LVP and dP/dt (Wash I). After approximately 3 min of Ca-free washout at which time LVP had decayed to less than 5 mm Hg, the heart was gas perfused for approximately 30 sec, and then perfused for approximately 3 min with a ca-containing K-H solution (Uptake) which resulted in restoration of contractile activity. The heart was again gas perfused prior to a second Ca-free washout (Wash II). If the perfusion protocol described above represented the first part of a cross-over experiment (see MEHTODS), the heart was then perfused with a Ca-containing K-H solution for at least 30 min to allow full recovery of LVP. After recovery, the heart was subjected to perfusion for 3 min with hypoxic K-H solution prior to Ca-free washout (Wash I), Ca-uptake (Uptake) and Ca-free washout (Wash II) with hypoxic K-H solutions. If the hearts had first been perfused with the hypoxic media, then in the second part of the cross-over design, the hearts were perfused with a similar protocol with normoxic K-H solutions. At the conclusion of the experiment, the hearts were gas perfused to clear the coronary vessels of perfusion fluid, removed from the perfusion apparatus, blotted dry, weighed and

Figure 2: Record of Left Intraventricular Pressure and dP/dt During a Typical Experiment.

Panel A: The upper tracing (LVP) is left intraventricular pressure. Lower record is dP/dt in a typical experiment with normoxic perfusion. After 30 min of equilibration with K-H solution, the heart was briefly gas-perfused followed by perfusion with a Ca-free K-H solution (WashI) for 3 min which resulted in reduction of LVP and dP/dt.

Panel B: Reperfusion with K-H solution (Uptake) after gas-perfusion resulted in rapid restoration of LVP and dP/dt. A second Ca-free washout (Wash II) after a brief gas perfusion resulted in reduction of LVP and dP/dt.


A
LVP
(mm Hg)
60
30
0
dP/dt
(arbitrary
units)
+1
0
-1
Equilibration
30 Min
Gas
Wash I
3 Min
Gas
B
LVP
(mm Hg)
60
30
0
dP/dt
(arbitrary
units)
+1
0
-1
Uptake
3 Min
Gas
Wash II
3 Min

ashed overnight for the determination of residual tissue Ca.

A. Typical Ca-free washout.

A typical Ca-free washout curve with a Ca-free normoxic K-H solution is shown on the upper part of Figure 3. Compartmental analysis of the curve by the unbiased statistical method yielded three components. These components have been arbitrarily labelled as Ca_I, Ca_{II} and Ca_{III} (Bailey and Dresel, 1968). The relatively rapid washout component, Ca_I, represents Ca washed out of the perfusion apparatus and the coronary vasculature. The halftime ($T_{1/2}$) for the washout of Ca from this component averaged 4 sec. A second less labile component, Ca_{II}, with a halftime of approximately 12 sec in normoxic hearts has been shown to be directly related to the maintenance of contractile force in the heart (Bailey and Dresel, 1968). The last washout component, Ca_{III}, had a halftime of approximately 95 sec under normal conditions.

The curve describing the decay of dP/dt during the Ca-free washout is shown on the lower part of Figure 3. Graphical analysis of the decay curve yielded two components. These components have been labelled arbitrarily as $dP/dt_{(1)}$ and $dP/dt_{(2)}$. The halftime for the decay of $dP/dt_{(1)}$ was found in this study to be highly correlated to the halftime for the washout of Ca from Ca_{II} in normoxic ($r= 0.75$; Figure 4, Table 2) as well as hypoxic hearts ($r= 0.74$; Table 2). The halftime for the decay of $dP/dt_{(2)}$ was also found to be significantly correlated to the washout of Ca from Ca_{III} under normoxic ($r= 0.59$; Table 2) as well as hypoxic ($r= 0.58$; Table 2) perfusions. This strongly suggests that both Ca_{II} and Ca_{III} are required for the development and maintenance of contractile activity in the heart. Total dP/dt in the normoxic hearts was 1650 mm Hg/sec of which 1350 ± 135 mm Hg/sec was attributed to $dP/dt_{(1)}$.

Figure 3: Washout of Ca and Decay of dP/dt During Normoxic Ca-free Washout (Wash I).

Panel A: The upper curve represents a typical Ca washout curve. The concentration of Ca measured in the effluent samples is plotted logarithmically as a function of time. The smooth curve joining the data points represents a reconstruction by addition of the least squares components, i.e. $[Ca]_t = Ca_I + Ca_{II} + Ca_{III}$. The dashed lines re-represent the least squares components resolved by compartmental analysis.

Panel B: The decay of dP/dt during Ca-free perfusion of the same heart. The dashed lines represent the components, $dP/dt_{(1)}$ and $dP/dt_{(2)}$ resolved by compartmental analysis. The smooth curve represents reconstruction by addition of the least squares components.

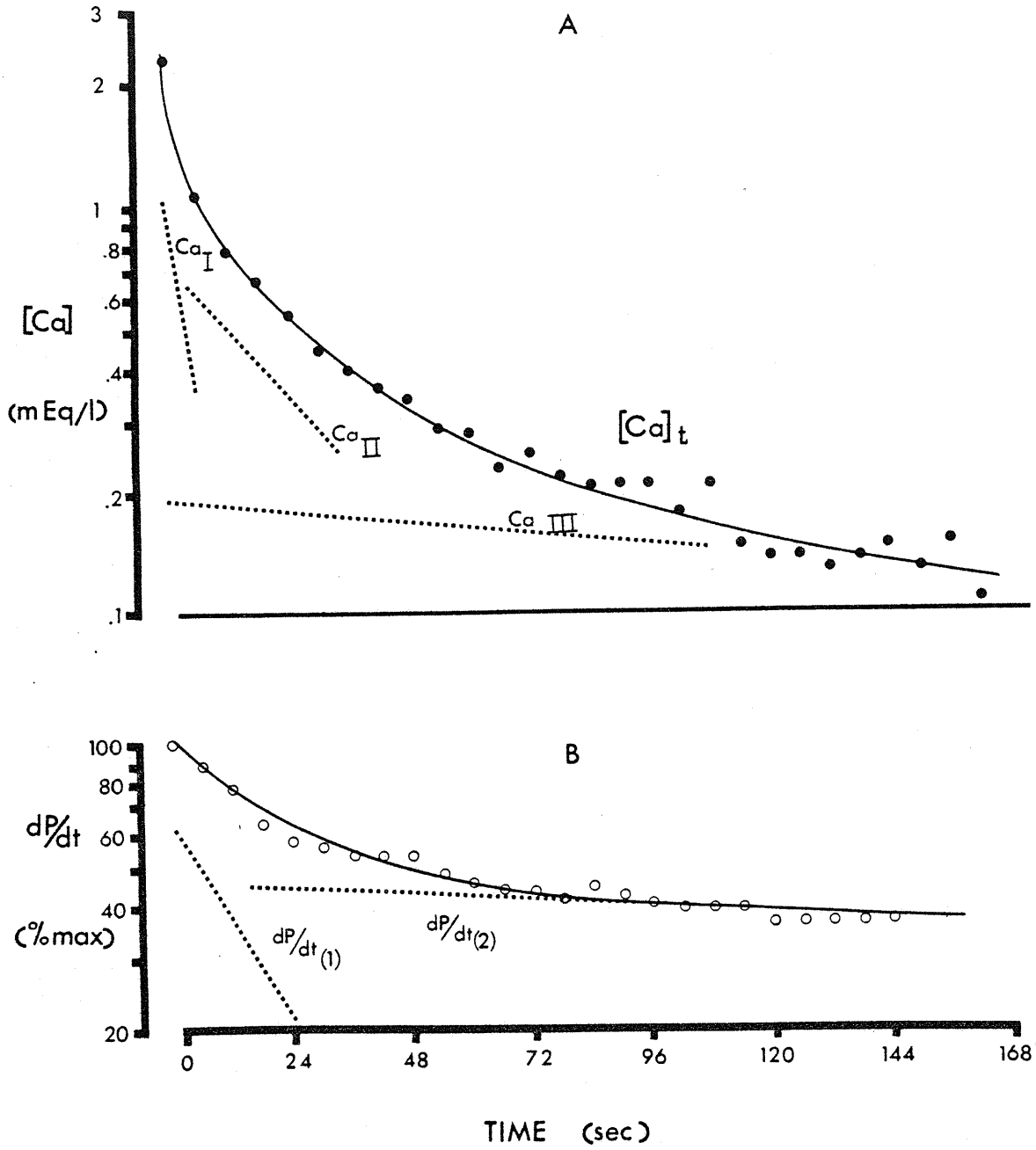

A
3
2
1
.8
.6
.4
.2
.1
[Ca]
(m Eq/l)
Ca I
Ca II
Ca III
[Ca]t
B
100
80
60
40
20
dP/dt
(%max)
dP/dt(1)
dP/dt(2)
0
24
48
72
96
120
144
168
TIME (sec)

Figure 4: The relationship between the halftime ($T_{1/2}$) for the decay of $dP/dt_{(1)}$ and the washout of Ca from Ca_{II}. The solid line indicates the least squares best fit straight line ($r=0.75$. $p< 0.05$). The correlation coefficient of the relationship did not differ significantly from unity.

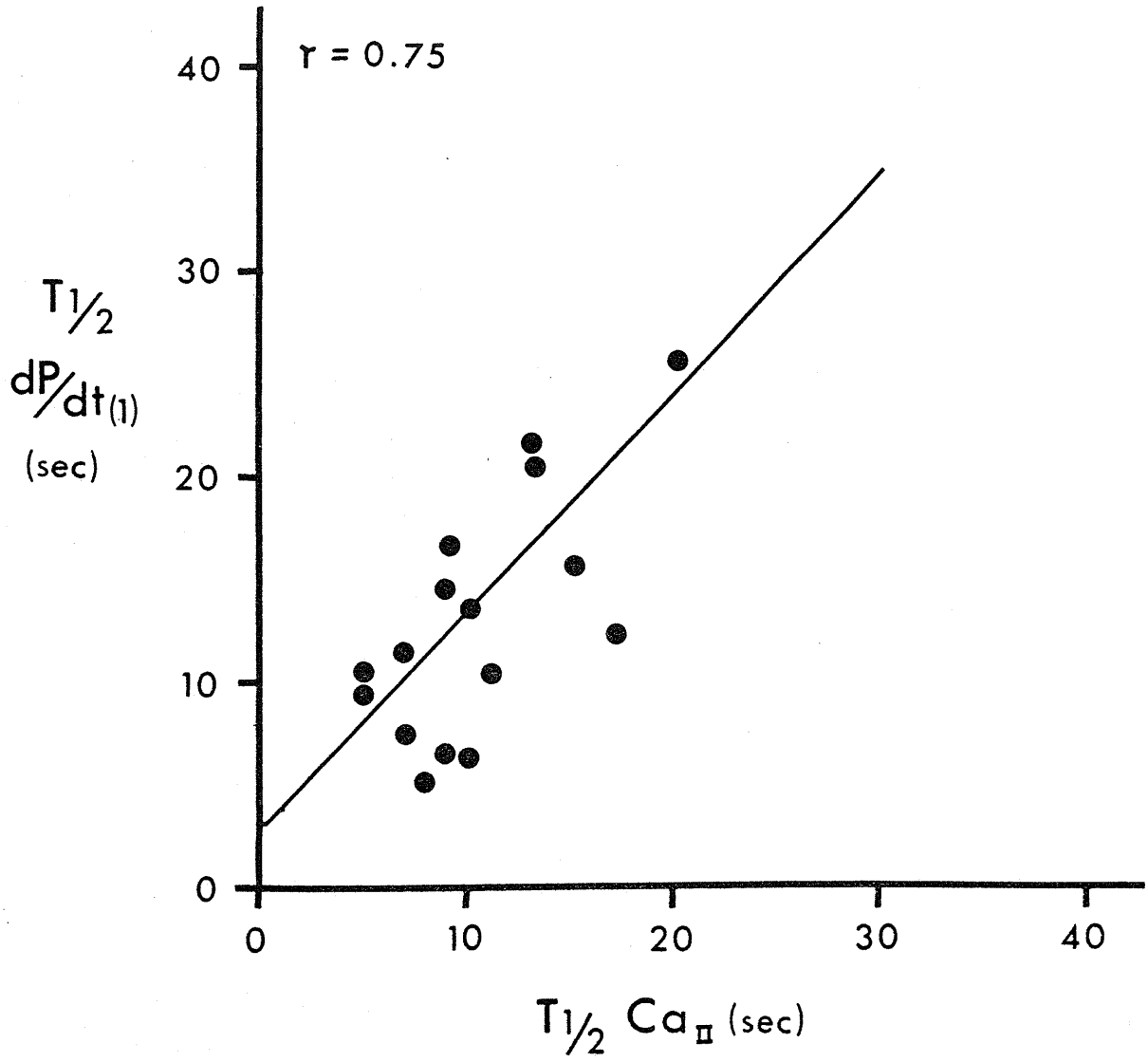

r = 0.75
40
30
20
10
0
0
10
20
30
40
T1/2 dP/dt(1) (sec)
T1/2 CaII (sec)

TABLE 2

Correlation between Halftime of Decay of the Two Components of dP/dt, $dP/dt_{(1)}$ and $dP/dt_{(2)}$ and Halftime for the Washout of Ca from Ca_{II} and Ca_{III}

	$T_{1/2}$		Coefficient of correlation(r)	
			Normoxia	Hypoxia
Ca_{II} : $dP/dt_{(1)}$		(8)	0.75[a]	0.74[a]
Ca_{III} : $dP/dt_{(2)}$		(8)	0.59[a]	0.58[a]

Number in parenthesis indicates the number of hearts.

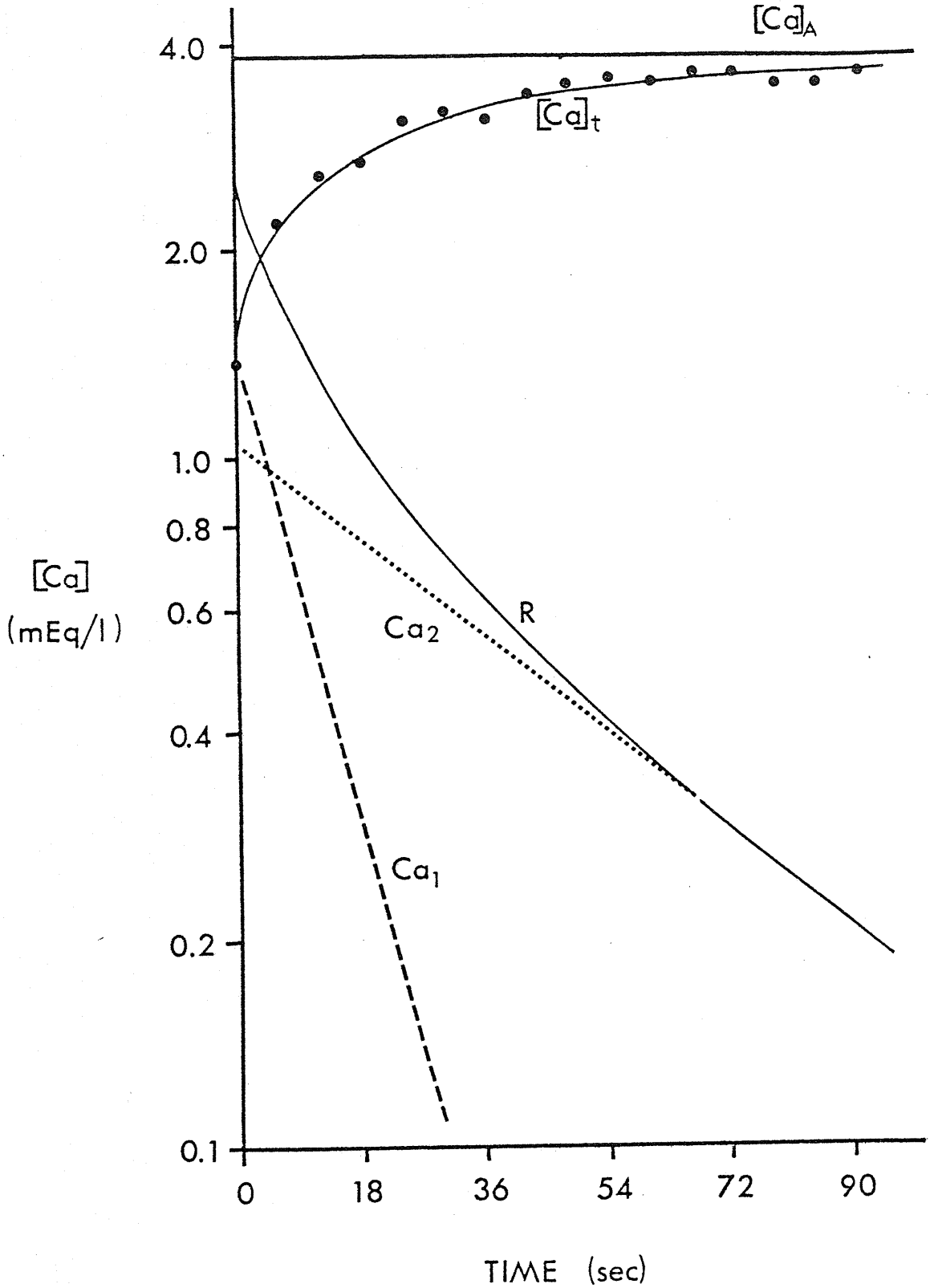
[a] Statistical significance ($p < 0.05$).

The slow component, $dP/dt_{(2)}$, contributed 300 $\pm$ 60 mm Hg/sec or less than 20% to contractility. Since none of the treatments in these experiments effected $dP/dt_{(2)}$ significantly, changes in contractility are reported as alterations in total dP/dt which primarily reflects changes in $dP/dt_{(1)}$.

B. Typical Ca-uptake.

Figure 5 shows a typical Ca-uptake curve during reperfusion of a heart depleted of Ca. The points indicate the concentration of Ca measured in each of the effluent samples. During reperfusion, the effluent Ca concentration gradually increased as the quantity of Ca extracted from the perfusate by the heart decreased with time, that is, as Ca content in the various tissue pools approached a steady state. After approximately 60 sec of reperfusion, the effluent Ca concentration became asymptotic to the perfusate Ca concentration. Subtraction of Ca concentration in the effluent from concentration of Ca in the perfusate yielded data points fitted by the curve R, representing concentration of Ca extracted into the heart from the perfusate. Graphical compartmental analysis of curve R by the unbiased statistical routine described in full in the METHODS section yielded two monoexponential components, Ca_1 and Ca_2. The fast uptake component, Ca_1, with a halftime of approximately 6 sec has been shown in Ca-uptake experiments to be essential to the process of coupling excitation to contraction in the heart (Bailey and Ong, 1974), that is, Ca which enters Ca_1 in some way mediates the release of Ca from Ca_2 or Ca_{II}, the released Ca then activates the contractile elements to initiate contraction. The slow uptake component, Ca_2, with a halftime of approximately 80 sec represents, at least in

Figure 5: Uptake of Ca in a typical experiment during reperfusion with a normoxic K-H solution after depleting the heart of Ca by Wash I. The concentration of Ca in the effluent is plotted logarithmically as a function of time. The horizontal line, Ca_A represents the concentration of Ca measured in the perfusion medium. The curve R is a reconstructed curve based on summation of the two least squares best fit components Ca_1 and Ca_2 resolved by graphical analysis. Curve R represents the difference between the concentration of Ca measured in the perfusion medium and the effluent. The curve $[Ca]_t$ is a reconstructed curve for the data points and was reconstructed basing on difference between Ca_A and curve R (See METHODS for full description of procedure).


[Ca]A
4.0
[Ca]t
2.0
1.0
0.8
[Ca]
(mEq/l)
0.6
R
Ca2
0.4
Ca1
0.2
0.1
0
18
36
54
72
90
TIME (sec)

part, Ca contained in Ca_{II} and perhaps in Ca_{III} of Ca washout data (see DISCUSSION) and is responsible for the restoration of contractile force in the heart.

II. The Effect of Hypoxia.

A. Oxygen extraction.

Oxygen tension in the perfusate and in the effluent during perfusion of hearts with normoxic and hypoxic K-H solutions was measured with an oxygen electrode. The results are shown in Table 3. Normoxic perfusate provided the heart with an oxygen tension (pO_2) of 617 mm Hg. The hearts extracted 83% of the oxygen from the normoxic K-H solution to yield a pO_2 of 104 mm Hg in the effluent. Hypoxic perfusate had a pO_2 of 41 mm Hg which was lower than pO_2 measured in the effluent during normoxic perfusion. The hearts perfused with this hypoxic perfusate only extracted 56% of the oxygen from the hypoxic K-H solution.

B. Perfusate flow.

Perfusate flow during perfusion with normoxic and hypoxic K-H solutions is shown in Table 4. Hypoxic perfusion significantly increased perfusate flow from 2.9 to 3.9 ml/min/g wet wt.

C. Contractile activity.

Figure 6 shows the reduction of contractile activity in a typical experiment caused by perfusion with a hypoxic K-H solution. In this experiment, hypoxic perfusion caused an immediate reduction of LVP which reached 60% of control within 15 sec. The effect of 3 min hypoxic perfusion on dP/dt is shown in Table 5. Three min of hypoxic perfusion resulted in a significant reduction of dP/dt to 64% of control.

TABLE 3

Oxygen Extraction in Hearts Perfused with Normoxic and Hypoxic K-H Solutions

	Perfusate pO_2 (mmHg)	Effluent pO_2 (mmHg)	Extraction (%)
Normoxia	617 ± 9[a](8)	104 ± 11(7)	83
Hypoxia	41 ± 1 (8)	18 ± 1(7)	56

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

TABLE 4

Perfusate Flow in Normoxic or Hypoxic Hearts in the Presence and Absence of Practolol

Treatment		Flow (ml/min/g wet wt)	
		Normoxia	Hypoxia
Untreated	(3)	2.9 ± 0.2[a]	3.9 ± 0.2[b]
practolol treated	(3)	3.2 ± 0.2	3.8 ± 0.2[b]

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

[b] Significantly different from untreated hearts in normoxia.

Figure 6: Changes in contractile force induced by hypoxic perfusion in the absence and presence of practolol.

Panel A: Immediate reduction of intraventricular pressure caused by perfusion with hypoxic K-H solution.

Panel B: Effect of practolol on the hypoxia-induced reduction of intraventricular pressure.

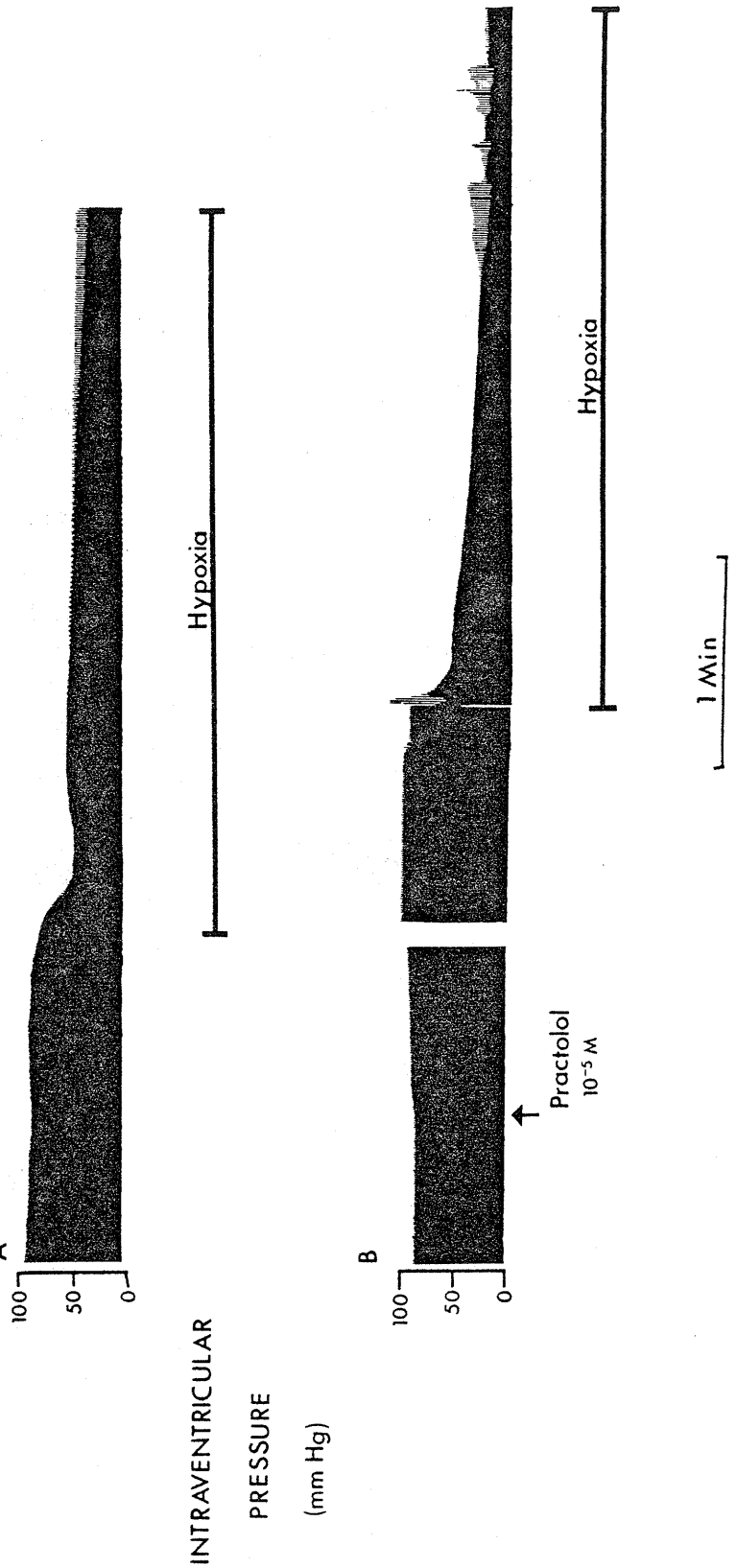

A
100
50
0
Hypoxia
INTRAVENTRICULAR
PRESSURE
(mm Hg)
B
100
50
0
Practolol
10^{-5} M
Hypoxia
1 Min

TABLE 5

The Effect of 3 min of Hypoxic Perfusion on dP/dt in the Absence and Presence of Practolol

Treatment		dP/dt (% control)	
		Normoxia	3 min of hypoxia
Untreated	(8)	100[a]	64.2 ± 5.7[b]
Practolol treated	(4)	128.3 ± 11.1[b]	59.0 ± 2.9[b]

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

[b] Significantly different from % dP/dt of control ($p < 0.05$).

D. Ca-free perfusion.

The halftime for the Washout of Ca from Ca_{II} and Ca_{III} is shown in Table 6. Neither 3 nor 10 min perfusion with the hypoxic medium altered the halftime for the washout of Ca from Ca_{II} or Ca_{III} (Comparison 1, see page 47 METHODS). The halftime for the decay of dP/dt from the fast and slow components, $dP/dt_{(1)}$ and $dP/dt_{(2)}$ are shown in Table 7. Hypoxia did not alter the rate of decay of the two components of contractile activity.

The intercept for the washout of Ca from Ca_{II} and Ca_{III} representing concentration of Ca washed out of these two Ca pools at zero time is shown in Table 8. Both 3 min and 10 min of hypoxic perfusion decreased the intercept for the washout of Ca from Ca_{II} and increased the intercept for the washout of Ca from Ca_{III}. However, only the increase of the intercept for the washout of Ca from Ca_{III} after 3 min of hypoxic perfusion was statistically significant.

The effect of 3 min hypoxic perfusion on Ca distribution in the two pools Ca_{II} and Ca_{III}, involved in E-C coupling is shown in Figure 7. The total quantity of Ca washed out of hearts during Wash I was not changed by 3 min hypoxic perfusion. However, 3 min of hypoxia resulted in a redistribution of approximately 0.6 mEq/kg tissue wet wt of Ca from Ca_{II} to Ca_{III} resulting in a significant reduction of Ca_{II} and a significant increase of Ca_{III} content. This redistribution of Ca was associated with a significant reduction of contractility in hypoxic hearts.

The effect of 10 min hypoxic perfusion on Ca distribution did not differ qualitatively from that measured after 3 min hypoxic perfusion (Figure 8). Ten min of hypoxic perfusion again did not affect the total

TABLE 6

The Effect of 3 and 10 min of Hypoxic Perfusion on the Halftime ($T_{1/2}$) for the Washout of Ca from Ca_{II} and Ca_{III}

			$T_{1/2}$ (sec)	
			Ca_{II}	Ca_{III}
Wash I :	Normoxia	(8)	11.9 ± 1.5[a]	79 ± 12
	Hypoxia		11.7 ± 1.5	93 ± 12
Wash II:	Normoxia	(8)	13.0 ± 4.3	118 ± 52
	Hypoxia		12.1 ± 4.3	110 ± 52

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

TABLE 7

The Effect of 3 and 10 min Hypoxic Perfusion on the Halftime ($T_{1/2}$) of Decay of $dP/dt_{(1)}$ and $dP/dt_{(2)}$

			$T_{1/2}$ (sec)	
			$dP/dt_{(1)}$	$dP/dt_{(2)}$
Wash I :	Normoxia	(8)	12.2 ± 6.3[a]	74 ± 14
	Hypoxia		18.5 ± 6.3	85 ± 14
Wash II:	Normoxia	(8)	15.1 ± 4.0	138 ± 21
	Hypoxia		18.6 ± 4.0	119 ± 21

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

TABLE 8

The Effect of 3 and 10 min Hypoxic Perfusion on the Intercept for the Washout of Ca from Ca_{II} and Ca_{III}

			Intercept (mEq/l)	
			Ca_{II}	Ca_{III}
Wash I :	Normoxia	(8)	1.84 ± 0.34[a]	0.52 ± 0.08
	Hypoxia		1.05 ± 0.34	0.82 ± 0.08[b]
Wash II:	Normoxia	(8)	1.41 ± 0.52	0.68 ± 0.09
	Hypoxia		1.15 ± 0.52	0.86 ± 0.09

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

[b] Significantly different from contral ($p < 0.05$).

Figure 7: The effect of 3 min hypoxic perfusion on the quantity of Ca in Ca_{II} and Ca_{III} from Wash I of eight normoxic (N) and hypoxic (H) hearts. Vertical bars represent S.E.M. * differs significantly from normoxic hearts ($p < 0.05$).

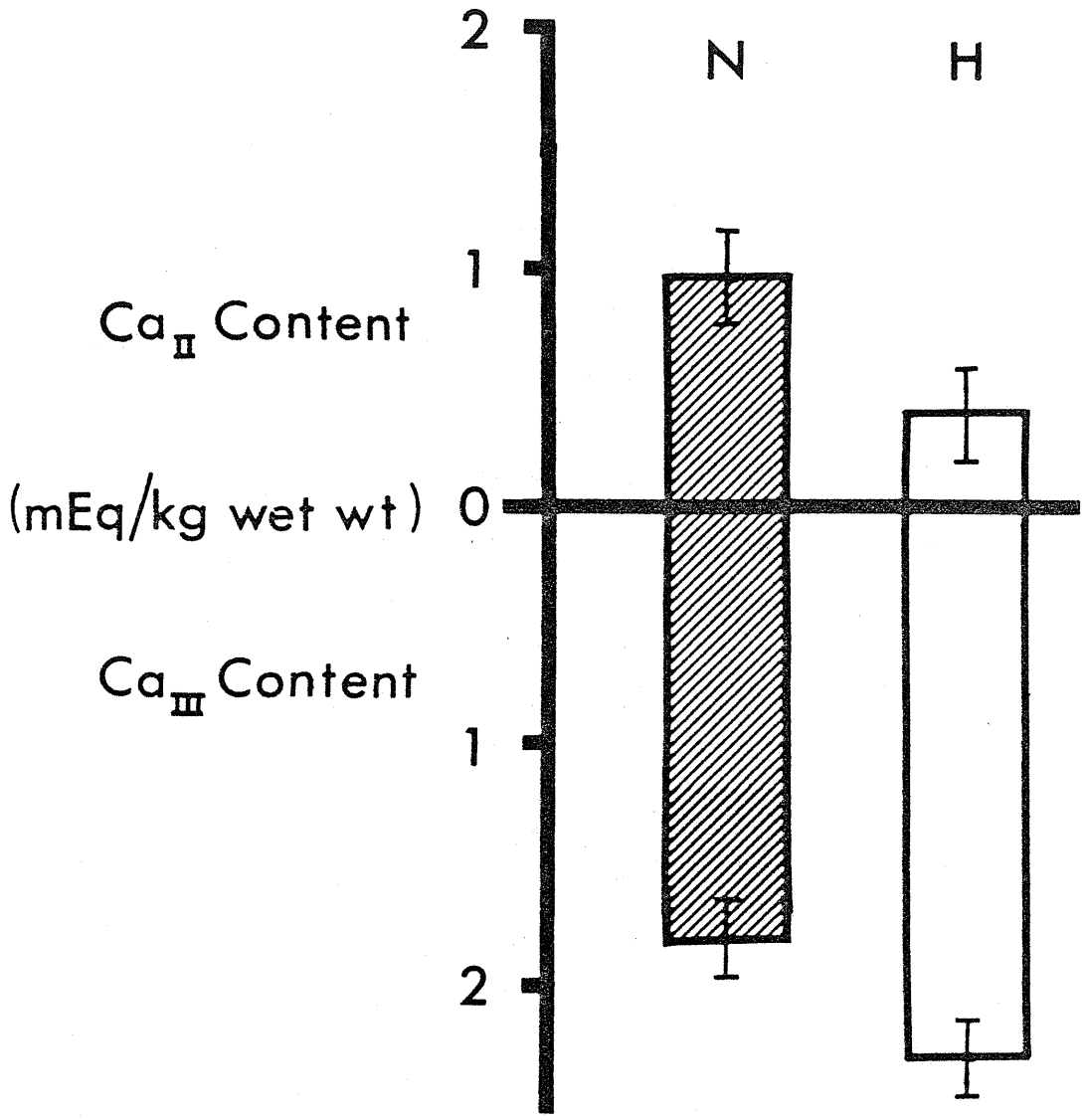
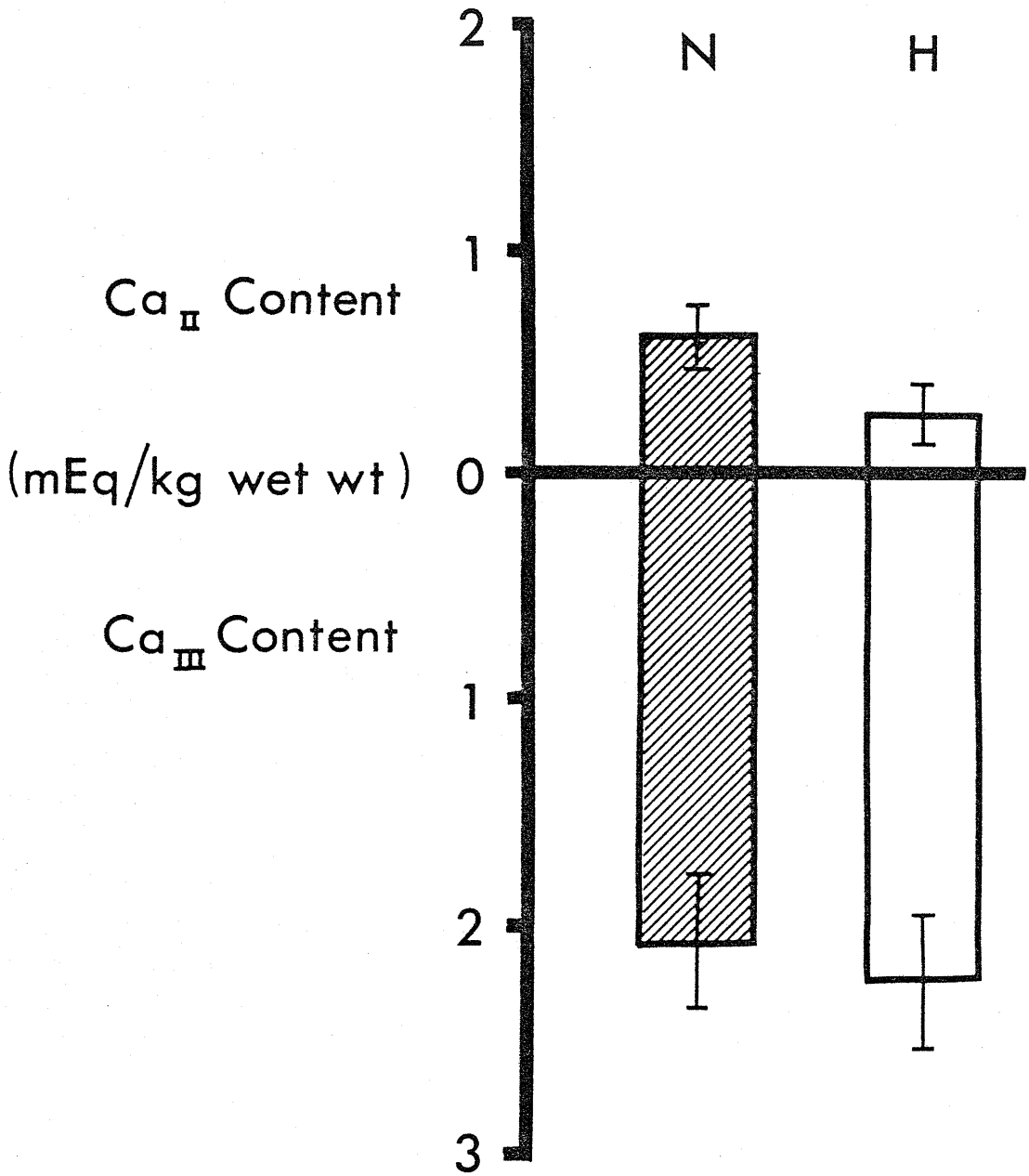

2
N
H
1
Ca_{II} Content
(mEq/kg wet wt)
0
Ca_{III} Content
1
2

Figure 8: The effect of 10 min hypoxic perfusion on the quantity of Ca in Ca_{II} and Ca_{III} from Wash II of eight normoxic (N) and hypoxic hearts (H). Vertical bars represent S.E.M. * differs significantly from normoxic hearts ($p < 0.05$).


N
H
2
1
0
1
2
3
Ca_{II} Content
(mEq/kg wet wt)
Ca_{III} Content

quantity of Ca washed out of the heart during Wash I. Similar to 3 min of hypoxic perfusion, Ca_{II} content was significantly reduced. However, the corresponding increase in Ca_{III} content was not statistically significant. Graphical analysis of ^{45}Ca washout curves obtained from Wash II did not yield results different from the washout of Ca (Figure 9), that is, 10 min of hypoxic perfusion resulted in a significant reduction of $^{45}Ca_{II}$ content, but the increase of $^{45}Ca_{III}$ content was not significant.

E. Ca-uptake.

The halftimes for the uptake of Ca are shown in Table 9. Although 6 min of hypoxic perfusion reduced the halftimes for the uptake of Ca by Ca_1 and Ca_2, the reductions were not statistically significant. The intercepts obtained by graphical analysis of the difference curve, R (see METHODS), are also shown in Table 9. Six min of hypoxic perfusion did not affect the intercept for the uptake of Ca by Ca_1 or Ca_2.

The effect of 6 min hypoxic perfusion on the content of Ca taken up by Ca_1 and Ca_2 is shown in Table 10. Hypoxia caused a three-fold reduction of the quantity of Ca taken up by Ca_1 from 0.27 to 0.09 mEq/kg wet wt ($P < 0.05$). Ca uptake into Ca_2 was reduced but not significantly from 2.33 to 1.77 mEq/kg wet wt. Graphical analysis of ^{45}Ca uptake curves did not yield results different from the uptake of Ca and will not be presented.

F. Tissue Ca and mitochondrial Ca.

Figure 10 shows the effect of 10 min hypoxic perfusion on total Wash II Ca, and residual tissue Ca. Total Wash II Ca represents the total quantity of Ca washed out of Ca_{II} and Ca_{III}. Residual tissue Ca is the quantity of Ca which remained in the heart after Wash II. Total

Figure 9: The effect of 10 min hypoxic perfusion on the quantity of ^{45}Ca in Ca_{II} and Ca_{III} from Wash II of eight normoxic (N) and hypoxic hearts (H). Vertical bars represent S.E.M. * differs significantly from normoxic hearts ($p < 0.05$).

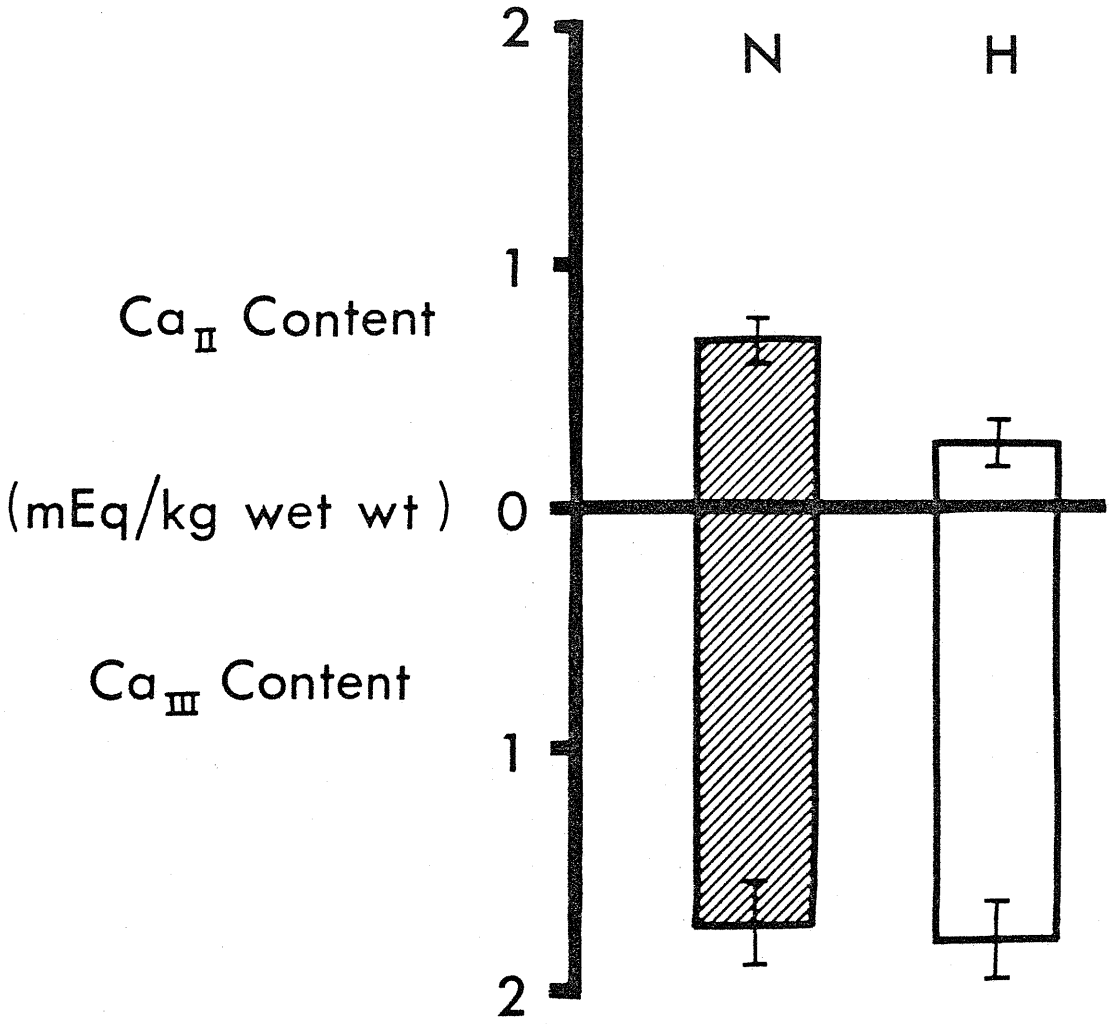

N
H
2
1
0
1
2
Ca_{II} Content
(mEq/kg wet wt)
Ca_{III} Content

TABLE 9

Effect of 6 min Hypoxic Perfusion on the Halftime ($T_{1/2}$) and Intercept for the Uptake of Ca into Ca_1 and Ca_2

	$T_{1/2}$ (sec)		Intercept (mEq/l)	
	Ca_1	Ca_2	Ca_1	Ca_2
Normoxia (4)	7.36 ± 1.36[a]	107 ± 49	1.02 ± 0.36	1.16 ± 0.09
Hypoxia	4.72 ± 1.36	79 ± 49	0.82 ± 0.36	1.26 ± 0.09

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

TABLE 10

Effect of 6 min Hypoxic Perfusion on the Quantity of Ca Taken up by Ca_1 and Ca_2

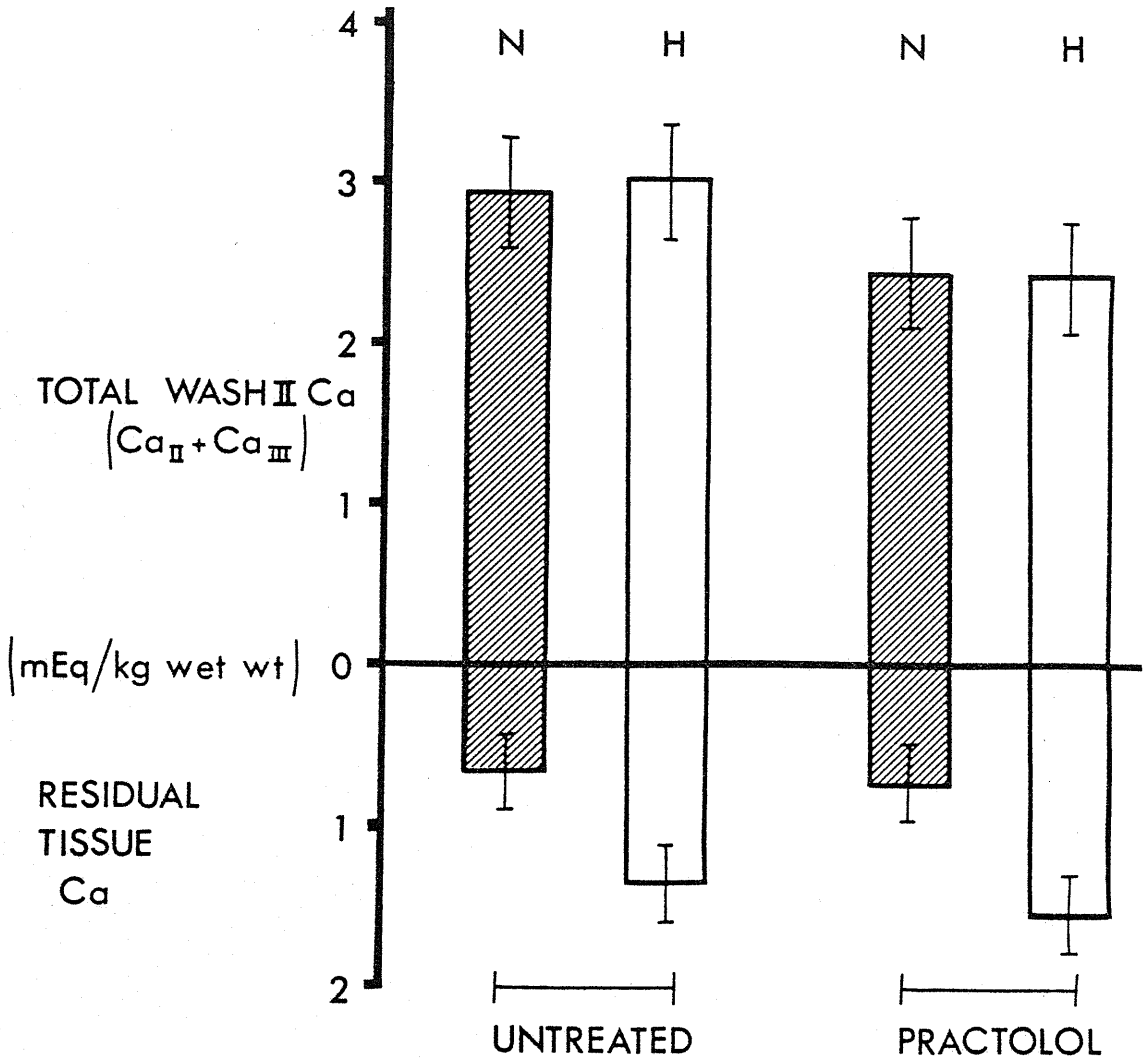
	Ca content (mEq/kg wet wt)	
	Ca_1	Ca_2
Normoxia (4)	0.27 ± 0.03[a]	2.33 ± 0.19
Hypoxia	0.09 ± 0.03[b]	1.77 ± 0.19

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

[b] Significantly different from control ($p < 0.05$).

Figure 10: The effect of 10 min hypoxic perfusion in the presence and absence of practolol (10^{-5}M) on total quantity of Ca washed out of the heart (total Wash II Ca) and quantity of Ca remained in the heart after Wash II (residual tissue Ca). ▨ represents the quantity of Ca washed out and that which remained after normoxic Ca-free washouts. ☐ represents the quantity of Ca washed out and that which remained after hypoxic Ca-free washouts. Number in parenthesis indicates the number of hearts. Vertical bars represent S.E.M. * differs significantly from normoxic hearts in the presence or absence of practolol.


4
3
2
1
0
1
2
N
H
N
H
TOTAL WASH II Ca
(Ca II + Ca III)
(mEq/kg wet wt)
RESIDUAL
TISSUE
Ca
UNTREATED
PRACTOLOL

tissue Ca is the sum of total Wash II Ca and residual tissue Ca. Ten min of hypoxic perfusion caused a 21% increase of total tissue Ca from 3.61 ± 0.39 mEq/kg wet wt to 4.38 ± 0.39 mEq/kg wet wt although this increase was not statistically significant. Determination of total tissue Ca in hearts subjected to 10 min of hypoxic perfusion without undergoing Ca-free washout or uptake showed that hypoxic perfusion caused a similar increase (27%) of total tissue Ca. The lower bars of Figure 10 show that 10 min hypoxic perfusion caused a 101% increase, from 0.67 to 1.35 mEq/kg wet wt, in the quantity of tightly bound and relatively non-exchangeable Ca remaining in hearts after Wash II. Ten min hypoxic perfusion increased mitochondrial Ca from 15.0 ± 2.9 to 17.9 ± 2.6 mEq Ca/100g protein, although this increase was not statistically significant.

III. The Effect of Practolol.

A. Perfusate flow.

The effect of practolol on perfusate flow is shown in Table 4. Practolol (10^{-5}M) did not increase perfusate flow in normoxic hearts. Perfusate flow in hypoxic hearts was increased to the same extent in the presence as well as the absence of practolol. It can therefore be concluded that practolol did not affect perfusate flow in normoxic or hypoxic hearts.

B. Contractile activity.

The effect of 10^{-5}M of practolol on contractile activity is shown in Figure 6. Practolol alone caused an immediate but slight increase in LVP and dP/dt. Treatment with practolol for 3 min increased LVP by 28% (Table 5). The drug, however, did not protect the heart against the re-

TABLE 11

Effect of Practolol on the Halftime ($T_{1/2}$) for the Washout of Ca from Ca_{II} and Ca_{III} in Normoxic hearts

		$T_{1/2}$ (sec)			
		Wash I		Wash II	
		Ca_{II}	Ca_{III}	Ca_{II}	Ca_{III}
Control	(4)	9.4 ± 2.1[a]	71 ± 23	12.2 ± 1.8	89 ± 13
Practolol	(4)	14.5 ± 3.4	152 ± 32	18.0 ± 2.8	68 ± 21

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

TABLE 12

Effect of Practolol on Halftime ($T_{1/2}$) for the Washout of Ca from Ca_{II} and Ca_{III} in Hypoxic Hearts.

		$T_{1/2}$ (sec)	
		Ca_{II}	Ca_{III}
Wash I : Practolol: (4)	Normoxia	12.4 ± 2.1[a]	101 ± 38
	Hypoxia	16.6 ± 2.1	92 ± 38
Wash II: Practolol: (4)	Normoxia	17.9 ± 2.4	67 ± 8
	Hypoxia	11.7 ± 2.4	77 ± 8

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

TABLE 13

Effect of Practolol on Halftime ($T_{1/2}$) of Decay of $dP/dt_{(1)}$ and $dP/dt_{(2)}$ in Hypoxic Hearts

			$T_{1/2}$ (sec)	
			$dP/dt_{(1)}$	$dP/dt_{(2)}$
Wash I : Practolol	Normoxia	(4)	19.5 ± 6.1[a]	68 ± 9
	Hypoxia		16.8 ± 6.1	106 ± 9
Wash II: Practolol	Normoxia	(4)	12.5 ± 3.8	59 ± 10
	Hypoxia		16.8 ± 3.8	96 ± 10

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

TABLE 14

Effect of Practolol on the Intercept for the Washout of Ca from Ca_{II} and Ca_{III} in Hypoxic Hearts

		Intercept (mEq/l)	
		Ca_{II}	Ca_{III}
Wash I : Practolol (4)	Normoxia	1.25 ± 0.39[a]	0.61 ± 0.18
	Hypoxia	1.06 ± 0.39	0.56 ± 0.18
Wash II: Practolol (4)	Normoxia	1.22 ± 0.11	0.46 ± 0.03
	Hypoxia	1.40 ± 0.11	0.61 ± 0.03[b]

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

[b] Significantly different from normoxic hearts ($p < 0.05$).

TABLE 15

Effect of Practolol on the Quantity of Ca Washed out of Ca_{II} and Ca_{III} in Normoxic Hearts

		Ca_{II} content (mEq/kg wet wt)		Ca_{III} content (mEq/kg wet wt)	
		Wash I	Wash II	Wash I	Wash II
Control	(4)	0.44 ± 0.12[a]	0.57 ± 0.13	1.50 ± 0.13	1.87 ± 0.29
Practolol	(4)	0.39 ± 0.16	0.53 ± 0.18	0.88 ± 0.19[b]	1.05 ± 0.40

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

[b] Significantly different from control hearts ($p < 0.05$).

from the first part of a cross-over design (Comparison 3). Treatment with practolol in normoxic hearts did not affect the quantity of Ca washed out of Ca_{II}, but reduced the quantity of Ca washed out of Ca_{III} in normoxic hearts. However, only the reduction of Ca_{III} content caused by 3 min of treatment with practolol was statistically significant ($p < 0.05$).

In the absence of practolol, hypoxic perfusion for 3 or 10 min resulted in a significant reduction in Ca_{II} content (Figures 7, 8 and 9). Practolol however, prevented the reduction of Ca_{II} content caused by 3 min hypoxic perfusion (Figure 11, Comparison 2). Although there was still a reduction in Ca_{II} content caused by 10 min hypoxic perfusion, the reduction was no longer statistically significant (Figure 12). In practolol-treated hearts the quantity of Ca washed out of Ca_{III} during either 3 or 10 min of hypoxic perfusion was not different from that during normoxic perfusion (Figures 11 and 12, Comparison 2). Hence, practolol prevented the elevation of Ca_{III} content associated with 3 or 10 min of hypoxic perfusion (Figures 7, 8 and 9, Comparison 4). Results obtained from analysis of ^{45}Ca washout curves were not different from that of Ca washout curves.

D. Ca-uptake.

The halftime for the uptake of Ca in practolol-treated hearts is shown in Table 16. Hypoxic perfusion did not affect the halftime for the uptake of Ca into Ca_1 or Ca_2 in practolol-treated hearts.

The intercept which indicates the quantity of Ca extracted from the perfusate by practolol-treated and Ca-depleted hearts at time zero is shown in Table 16. Practolol did not affect the lack of effect of

Figure 11: The effect of 3 min hypoxic perfusion on the quantity of Ca in Ca_{II} and Ca_{III} from Wash I of eight normoxic (N), practolol-treated and hypoxic (H), practolol-treated hearts. Vertical bars represent S.E.M.

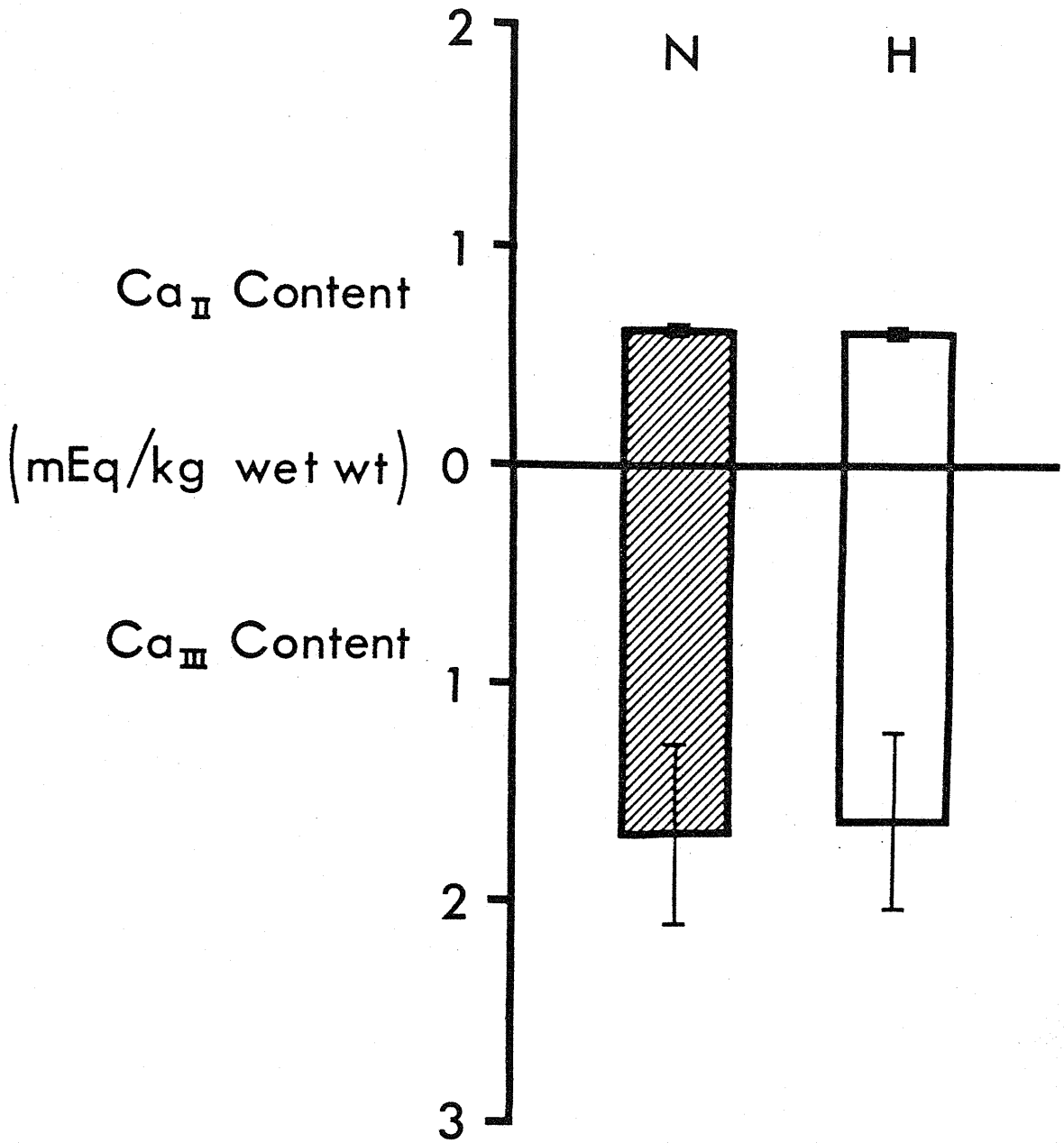

2
N
H
1
Ca_{II} Content
(mEq/kg wet wt)
0
Ca_{III} Content
1
2
3

Figure 12: The effect of 10 min hypoxic perfusion on the quantity of Ca in Ca_{II} and Ca_{III} from Wash II of eight normoxic (N), practolol-treated and hypoxic (H), practolol-treated hearts. Vertical bars represent S.E.M.

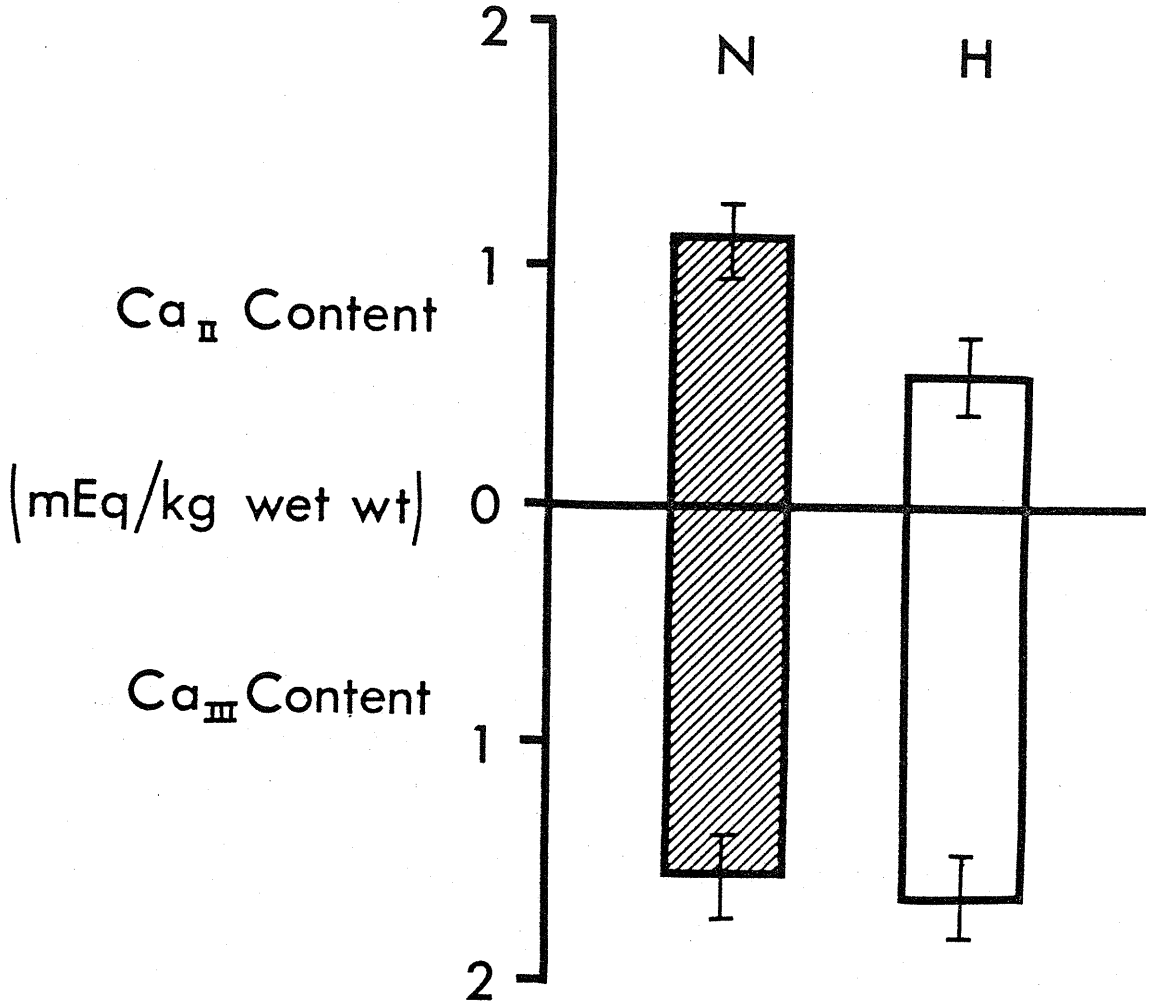

2
N
H
1
Ca_{II} Content
(mEq/kg wet wt)
0
Ca_{III} Content
1
2

TABLE 16

The Effect of Practolol on the Halftime ($T_{1/2}$) and Intercept for the Uptake of Ca by Ca_1 and Ca_2 in Hypoxic Hearts

		$T_{1/2}$ (Sec)		Intercept (mEq/l)	
		Ca_1	Ca_2	Ca_1	Ca_2
Practolol:	Normoxia (4)	6.7 ± 1.6[a]	69 ± 24	0.60 ± 0.20	0.87 ± 0.13
	Hypoxia	4.6 ± 1.6	68 ± 24	0.86 ± 0.20	1.05 ± 0.13

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

hypoxic perfusion on the intercept for the uptake of Ca into Ca_1 and Ca_2.

The effect of practolol on Ca uptake in normoxic hearts is shown in Table 17. Values used for the comparison consisted of data obtained from the first part of a cross-over design (Comparison 3). Practolol significantly reduced the content of Ca taken up by Ca_1. The content of Ca taken up by Ca_2 was also reduced but this reduction was not statistically significant.

The effect of practolol on Ca uptake in hypoxic hearts is shown in Table 18. The content of Ca taken up by Ca_1 in practolol-treated hearts after 6 min hypoxic perfusion was reduced although the reduction was not statistically significant. There was a slight reduction of the content of Ca taken up by Ca_2 after 6 min hypoxic perfusion. Thus these results indicate that although practolol prevented the reduction of Ca_{II} content caused by 3 or 10 min of hypoxic perfusion in Wash I and II (Figure 11 and 12) it has little effect on the reduction of Ca uptake by Ca_1 and Ca_2 in hypoxic hearts (Comparison 4).

E. Tissue Ca.

The effect of practolol on total Wash II Ca, residual tissue Ca and total tissue Ca is shown in Figure 10. Practolol did not affect total Wash II Ca, residual tissue Ca or total tissue Ca in hearts perfused with normoxic media or with hypoxic media for 10 min (Comparison 4). This indicates that even in the presence of practolol, the quantity of tightly bound and relatively non-exchangeable Ca was increased by 10 min hypoxic perfusion. Hence, practolol was unable to protect the heart against the hypoxia induced increase of residual tissue Ca.

F. Restoration of dP/dt after hypoxia.

Figure 13 shows the reduction of dP/dt caused by 3 min hypoxic

TABLE 17

The Effect of Practolol on Ca Uptake by Ca_1 and Ca_2 in Normoxic Hearts

		Ca content (mEq/kg wet wt)	
		Ca_1	Ca_2
Control	(4)	0.26 ± 0.02[a]	2.71 ± 0.50
Practolol	(2)	0.09 ± 0.03[b]	1.24 ± 0.71

Number in parenthesis indicates the number of hearts.

[a] Mean $\pm$ S.E.

[b] Significantly different from control ($p < 0.05$).

TABLE 18

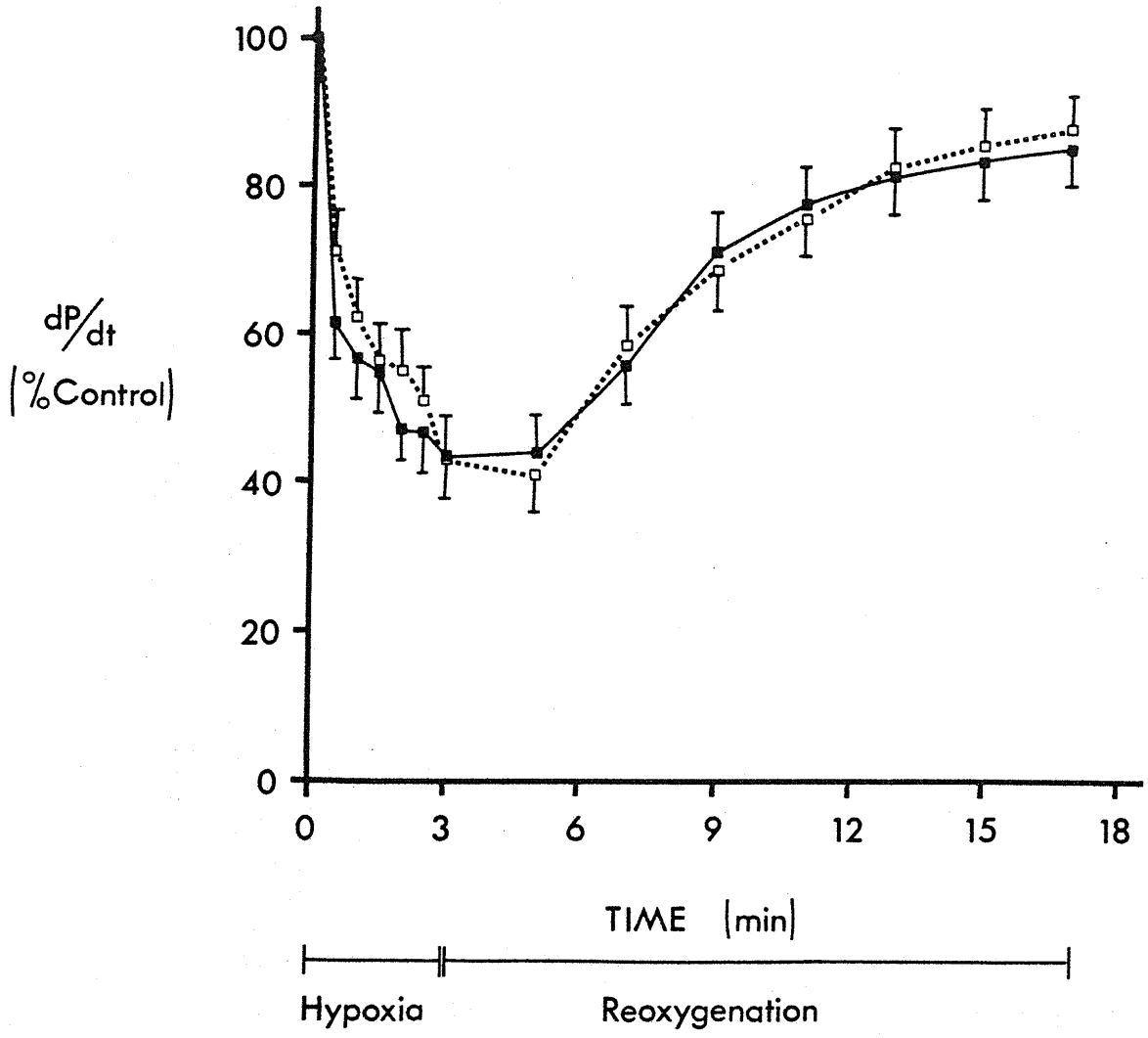
The Effect of Practolol on Ca Uptake by Ca_1 and Ca_2 after 6 min Hypoxic Perfusion

		Ca content (mEq/kg wet wt)	
		Ca_1	Ca_2
Practolol:	Normoxia (4)	0.14 ± 0.04[a]	1.57 ± 0.27
	Hypoxia	0.08 ± 0.04	1.20 ± 0.27

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

Figure 13: The effect of practolol on the restoration of dP/dt after 3 min of hypoxic perfusion. The broken line (▪▪▪▪▪▪) represents % dP/dt in the absence of practolol and the (——) solid line represents % dP/dt in practolol-treated hearts. Vertical bars represents Mean ± S.E.


100
80
60
40
20
0
dP/dt
(%Control)
0
3
6
9
12
15
18
TIME (min)
Hypoxia
Reoxygenation

perfusion and the restoration of dP/dt by normoxic perfusion in the presence and absence of practolol. Practolol (10^{-5}M) did not protect the heart against the reduction of dP/dt caused by hypoxic perfusion since dP/dt was reduced to approximately 43% of control in both the untreated and practolol treated hearts. The drug also did not change the rate of restoration of dP/dt upon reoxygenation.

Practolol had a similar lack of protective effect on the reduction of dP/dt caused by 10 min hypoxic perfusion and on subsequent restoration of dP/dt with normoxic perfusion (Figure 14). During 10 min hypoxic perfusion dP/dt was actually reduced to a lower level in the presence of practolol. Practolol again did not increase the rate of restoration of dP/dt. The reduction of dP/dt was reversible since continued perfusion with a normoxic K-H solution for 30 min restored dP/dt to levels not different from control.

IV. The Effect of Isoproterenol.

A. Contractile activity.

Isoproterenol was infused at the rate sufficient to cause a 70-100% increase in LVP. This was called arbitrarily low dose treatment with isoproterenol which represents a range of concentrations from 2.4×10^{-8}M to 1.2×10^{-7}M with a mean of 5.2×10^{-8}M. In the typical experiment shown in Figure 15, infusion of isoproterenol at a rate which produced a final drug concentration of 2.4×10^{-8}M resulted in a 75% increase in LVP. After stopping the infusion, contractile activity rapidly returned to control levels. In the same heart, infusion of practolol at 10^{-5}M resulted in a slight increase in LVP and dP/dt. This dose of practolol blocked the positive inotropic effect of low doses isoproterenol.

Figure 14: The effect of practolol on restoration of dP/dt after 10 min of hypoxic perfusion. The broken line (▪▪▪▪▪▪) represents % dP/dt in the absence of practolol and the (——) solid line represents % dP/dt in practolol-treated hearts. Vertical bar represents Mean ± S.E.

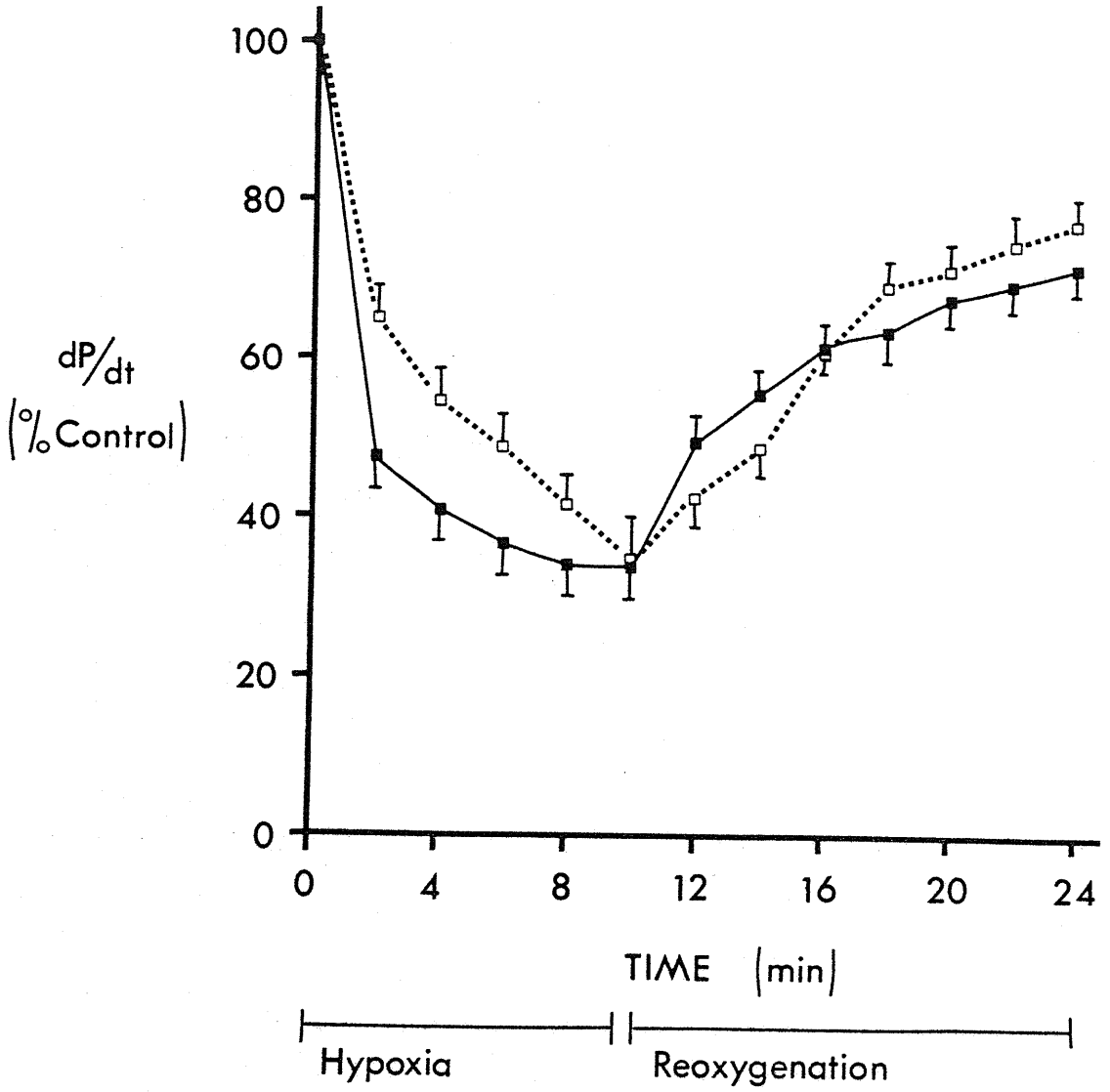
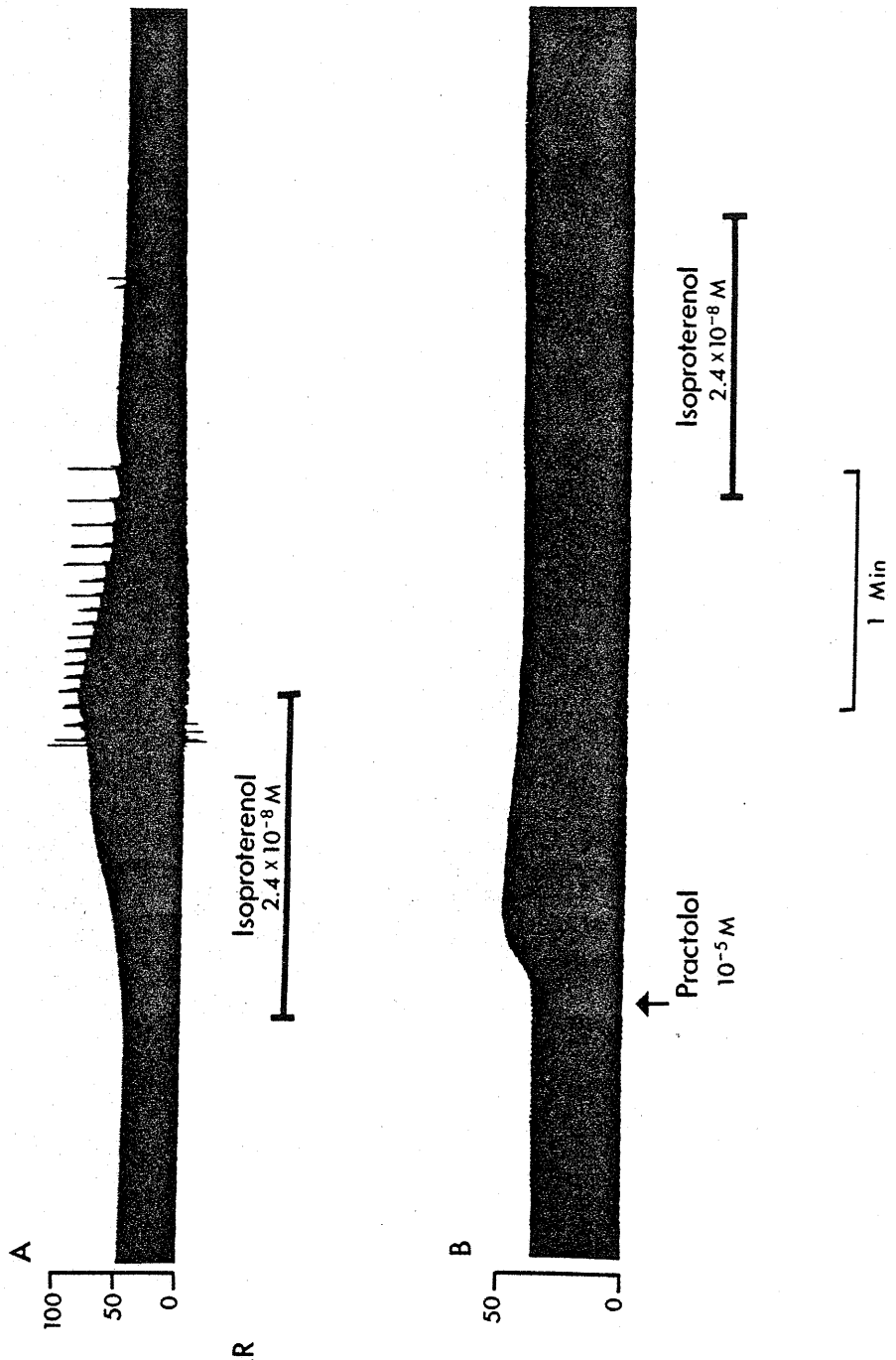

100
80
60
40
20
0
dP/dt
(% Control)
0
4
8
12
16
20
24
TIME (min)
Hypoxia
Reoxygenation

Figure 15: Record of left intraventricular pressure showing the positive inotropic effect of isoproterenol and blockade of this effect by practolol.

Panel A: Immediate increase of intraventricular pressure by isoproterenol.

Panel B: Blockade of the positive inotropic effect of isoproterenol by practolol. Note the significant intrinsic positive inotropic effect of practolol treatment.


A
100
50
0
INTRAVENTRICULAR
PRESSURE
(mm Hg)
Isoproterenol
2.4 x 10⁻⁸ M
B
50
0
Practolol
10⁻⁵ M
Isoproterenol
2.4 x 10⁻⁸ M
1 Min

Several hearts were treated with high doses of isoproterenol in an attempt to assess the acute effects which may lead to subsequent tissue necrosis. In these experiments the drug was infused at a rate to produce a 100-200% increase of LVP. The range of dosages used in the several experiments was from 1.3×10^{-7}M to 3.9×10^{-7}M with a mean dose of 2.6×10^{-7}M. Infusion of the drug was continued for 25 min before the start of Ca-free washout. Administration of both a low and high dose of isoproterenol caused the development of tachycardia and arrhythmia.

B. Ca-free washout.

The effect of isoproterenol on the halftime for the washout of Ca from Ca_{II} and Ca_{III} is shown in Table 19. Values used were taken from the first part of a cross-over design. The halftime for Ca washout from Ca_{II} was increased by infusion of both a low dose and a high dose of isoproterenol, although the increase after 6 min of treatment with a low dose of isoproterenol was not statistically significant. Isoproterenol did not affect the halftime for the washout of Ca from Ca_{III}. The halftime for the washout of Ca from Ca_{II} and Ca_{III} in hearts treated with a low dose of isoproterenol was not affected by treatment with practolol (Table 20).

Unlike the graphical analysis of the decay of dP/dt during Ca-free washout in normoxic, hypoxic or practolol-treated hearts, graphical analysis of the decay of dP/dt during Ca-free washout in isoproterenol-treated hearts in the absence as well as in the presence of practolol yielded a single monoexponential component (Figure 16). Comparison of the Ca-washout pattern in untreated and isoproterenol treated hearts (Figure 3) shows that the halftime for the washout of Ca from Ca_{II} was

TABLE 19

The Effect of Isoproterenol on the Halftime ($T_{1/2}$) for the Washout of Ca from Ca_{II} and Ca_{III}

		$T_{1/2}$ (sec)			
		Wash I		Wash II	
		Ca_{II}	Ca_{III}	Ca_{II}	Ca_{III}
Control:	(4)	9.4 ± 2.1[a]	71 ± 23	12.2 ± 1.8	89 ± 13
Isoproterenol:					
low dose	(3)	19.7 ± 2.7[b]	86 ± 26	15.3 ± 2.3	95 ± 17
high dose	(5)	25.6 ± 2.1[b]	140 ± 20	---	---

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

[b] Significantly different from control ($p < 0.05$).

TABLE 20

The Effect of Isoproterenol on the Halftime ($T_{1/2}$) for the Washout of Ca from Ca_{II} and Ca_{III} in the Presence and Absence of Practolol (10^{-5}M)

		$T_{1/2}$ (sec)	
		Ca_{II}	Ca_{III}
Wash I :	Isoproterenol: low dose (4)	18.9 ± 5.6[a]	119 ± 31
	Isoproterenol: low dose + Practolol	15.2 ± 5.6	84 ± 31
Wash II:	Isoproterenol: low dose (4)	15.5 ± 3.2	103 ± 31
	Isoproterenol: low dose + Practolol	21.9 ± 3.2	87 ± 31

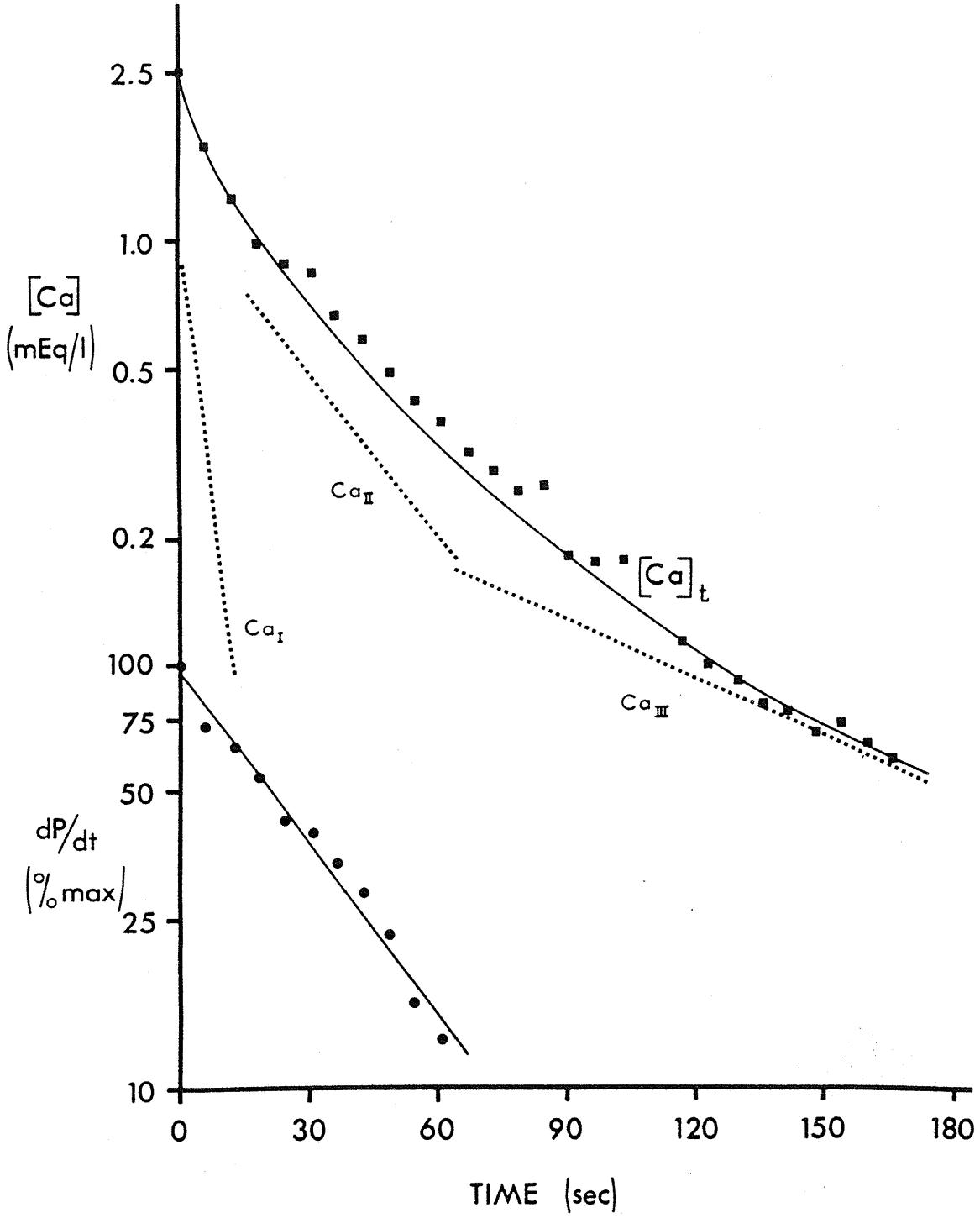
Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

Figure 16: Washout of Ca and decay of dP/dt during Ca-free perfusion in isoproterenol-treated hearts.

Panel A: The upper curve represents a typical Ca washout curve in hearts treated with a high dose of isoproterenol. The concentration of isoproterenol used in this particular heart was 1.8×10^{-7}M which resulted in a 200% increase of LVP. The concentration of Ca measured in the effluent samples is plotted logarithmically as a function of time. The smooth curve joining the data points represents a reconstruction by addition of the least squares components, i.e., $[Ca]_t = Ca_I + Ca_{II} + Ca_{III}$. The dashed lines represent the least squares components resolved by compartmental analysis (see METHODS).

Panel B: The decay of dP/dt during Ca-free perfusion of the same heart.


2.5
1.0
0.5
0.2
100
75
50
25
10
[Ca] (mEq/l)
dP/dt (%max)
Ca_I
Ca_{II}
Ca_{III}
$[Ca]_t$
0
30
60
90
120
150
180
TIME (sec)

increased by isoproterenol. The increased halftime for the washout of Ca from Ca_{II} by isoproterenol is further illustrated in Table 21. Since our results suggest that the washout of Ca from Ca_{II} may be responsible for the decay of $dP/dt_{(1)}$ and the washout of Ca from Ca_{III} may be responsible for the decay of $dP/dt_{(2)}$ (Table 2) it is possible that the failure to detect the decay of $dP/dt_{(2)}$ was due to increased halftime of Ca washout from Ca_{II}. Table 21 shows the halftime of decay of dP/dt in hearts treated with isoproterenol. The halftime of decay of dP/dt was not affected by treatment with practolol.

The intercept for the washout of Ca from Ca_{II} and Ca_{III} in hearts treated with isoproterenol alone or isoproterenol in the presence of practolol is shown in Table 22. Practolol reduced the intercept for the washout of Ca from Ca_{II} and increased the intercept for the washout of Ca from Ca_{III} in isoproterenol-treated hearts. However, only the practolol-induced reduction of Ca_{II} intercept after 6 min of treatment with isoproterenol and the increase of Ca_{III} intercept immediately after treatment with isoproterenol were statistically significant.

The effect of low doses of isoproterenol on Ca_{II} and Ca_{III} contents is shown in Table 23. The values reported represent the means of data obtained from the first half of a cross-over design. The immediate effect of a low dose of isoproterenol was a significant increase of the content of Ca contained in Ca_{II}, from 0.44 to 1.09 mEq/kg wet wt. This increase in Ca_{II} content was independent of the duration or dosage of isoproterenol used in the study, since a similar increase was obtained after 6 min of infusion of a low dose of isoproterenol, from 0.57 to 1.33 mEq/kg wet wt or after 25 min of infusion of a high dose of the drug, from 0.44 to 1.15 mEq/kg wet wt. Practolol at 10^{-5}M which blocked the

TABLE 21

The Effect of Practolol on Halftime ($T_{1/2}$) of Decay of dP/dt in Hearts Treated with Isoproterenol

			$T_{1/2}$: dP/dt (sec)
	Isoproterenol:	low dose	23.8 ± 1.8
		(4)	
Wash I:	Isoproterenol:	low dose + Practolol	22.5 ± 1.8
	Isoproterenol:	low dose	19.8 ± 6.7
		(4)	
Wash II:	Isoproterenol:	low dose + Practolol	28.8 ± 6.7

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

TABLE 22

The Effect of Practolol on the Intercept for the Washout of Ca from Ca_{II} and Ca_{III} in Hearts Treated with Isoproterenol

		Intercept (mEq/l)	
Wash I:	Isoproterenol: low dose	1.62 ± 0.36[a]	0.30 ± 0.02
	Isoproterenol: low dose (4) + Practolol	0.99 ± 0.36	0.44 ± 0.02[b]
Wash II:	Isoproterenol: low dose	1.57 ± 0.10	0.41 ± 0.01
	Isoproterenol: low dose (4) + Practolol	1.13 ± 0.10[b]	0.45 ± 0.01

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

[b] Significantly different from isoproterenol-treated hearts ($p < 0.05$).

TABLE 23

The Effect of Isoproterenol on the Washout of Ca from Ca_{II} and Ca_{III}

	Ca_{II} content (mEq/kg wet wt)		Ca_{III} content (mEq/kg wet wt)	
	Wash I	Wash II	Wash I	Wash II
Control: (4)	0.44 ± 0.12[a]	0.57 ± 0.13	1.50 ± 0.13	1.87 ± 0.29
Isoproterenol:				
low dose (3)	1.09 ± 0.13[b]	1.33 ± 0.15[b]	1.10 ± 0.15	1.69 ± 0.33
high dose (5)	1.15 ± 0.10[b]	---	0.96 ± 0.12[b]	---

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

[b] Statistically different from control hearts ($p < 0.05$).

positive inotropic effect of isoproterenol (Figure 15), was unable to prevent the elevation of Ca_{II} content caused by the immediate effect or 6 min of treatment with isoproterenol (Figures 17 and 18). That is, Ca_{II} content was increased by the immediate and 6 min treatment with isoproterenol, to 0.96 and 0.95 mEq/kg wet wt, respectively, in the presence of practolol.

The content of Ca washed out of Ca_{III} was slightly reduced by the immediate effect as well as 6 min of treatment with a low dose of isoproterenol (Table 23) although the reductions were not statistically significant. Treatment with a high dose of isoproterenol, however, significantly reduced the content of Ca washed out of Ca_{III} from 1.50 to 0.96 mEq/kg wet wt (Table 23). Practolol also had no effect on the content of Ca washed out of Ca_{III} in isoproterenol-treated hearts (Figures 17 and 18).

C. Ca-uptake.

The halftime of Ca uptake in isoproterenol-treated hearts is shown in Table 24. Practolol did not affect the halftime for the uptake of Ca by Ca_1 but significantly reduced the halftime for the uptake of Ca by Ca_2.

The intercept for the quantity of Ca extracted by isoproterenol-treated, Ca-depleted hearts is shown in Table 24. Practolol did not affect the intercept for the uptake of Ca by Ca_1 or Ca_2 in isoproterenol-treated hearts.

The effect of a low dose of isoproterenol on Ca-uptake is shown in Table 25. Values used for analysis of variance were taken from the first part of a cross-over design. Isoproterenol was found to significantly reduce the quantity of Ca extracted by Ca_1 when compared to nor-

Figure 17: The effect of practolol on the quantity of Ca in Ca_{II} and Ca_{III} from Wash I of four hearts treated with isoproterenol alone (I), or with isoproterenol and practolol (IP). Vertical bars represent S.E.M.

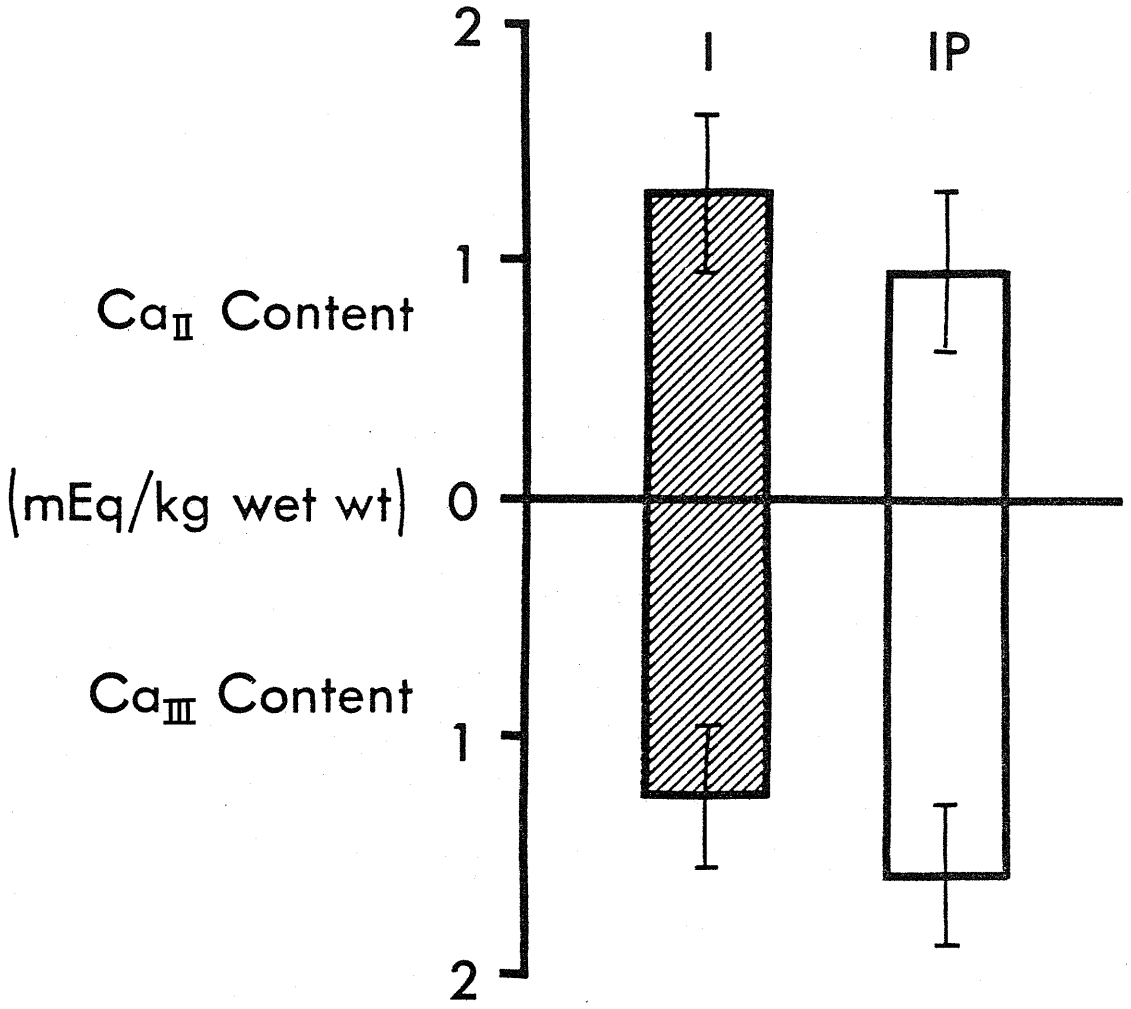

2
1
0
1
2
I
IP
Ca_{II} Content
(mEq/kg wet wt)
Ca_{III} Content

Figure 18: The effect of practolol on the quantity of Ca in Ca_{II} and Ca_{III} from Wash II of four hearts treated with isoproterenol alone (I), or with isoproterenol and practolol (IP). Vertical bars represent S.E.M.

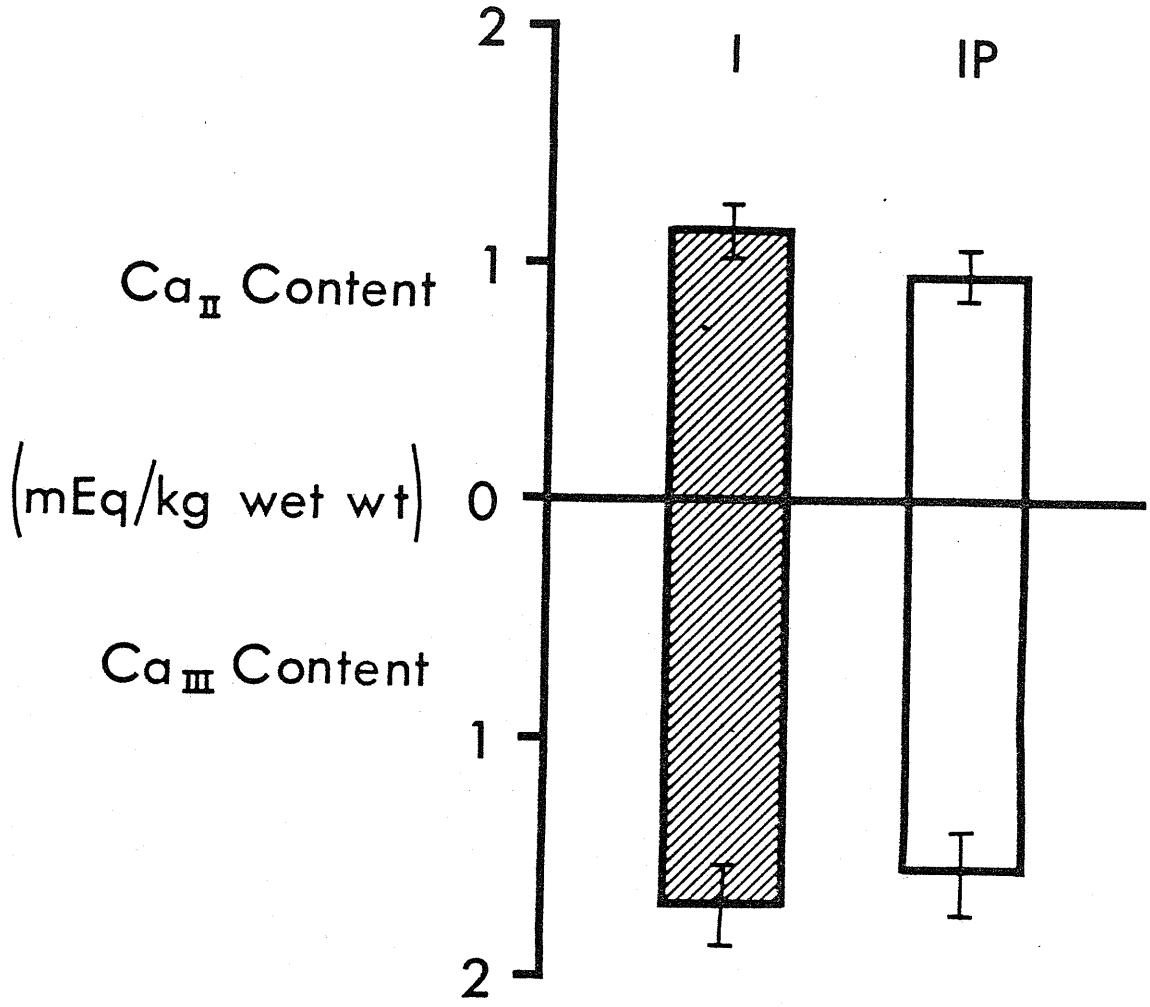

2
I
IP
Ca_{II} Content
1
(mEq/kg wet wt)
0
Ca_{III} Content
1
2

TABLE 24

The Effect of Practolol on the Halftime ($T_{1/2}$) and Intercept for Up-of Ca by Ca_1 and Ca_2 in Hearts Treated with Isoproterenol

	$T_{1/2}$ (sec)		Intercept (mEq/1)	
	Ca_1	Ca_2	Ca_1	Ca_2
Isoproterenol: low dose (4)	5.6 ± 0.8[a]	150 ± 16	0.97 ± 0.34	0.98 ± 0.10
Isoproterenol: low dose + Practolol	4.5 ± 0.8	96 ± 16[b]	0.85 ± 0.34	0.95 ± 0.10

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

[b] Significantly different from isoproterenol-treated hearts. ($p < 0.05$).

TABLE 25

The Effect of a Low Dose of Isoproterenol on Uptake of Ca by Ca_1 and Ca_2

	Ca content (mEq/kg wet wt)	
	Ca_1	Ca_2
Control (4)	0.26 ± 0.02[a]	2.71 ± 0.50
Isoproterenol (3)	0.14 ± 0.03[b]	3.37 ± 0.58

Number in parenthesis indicates the number of hearts.

[a] Mean $\pm$ S.E.

[b] Significantly different from control ($p < 0.05$).

moxic hearts. The quantity of Ca extracted by Ca_2 in isoproterenol-treated hearts was increased, but not significantly. The effect of practolol on Ca uptake in isoproterenol-treated hearts is shown in Table 26. Practolol was found to cause a significant reduction of the quantity of Ca taken by Ca_1. Practolol did not affect the quantity of Ca extracted by Ca_2.

D. Mitochondrial Ca.

Treatment for 25 min with a high dose of isoproterenol caused a significant reduction of mitochondrial Ca from 15.0 $\pm$ 2.9 to 5.8 $\pm$ 2.9 mEq Ca/100g protein.

TABLE 26

The Effect of Practolol on Uptake of Ca by Ca_1 and Ca_2 in Hearts Treated with Isoproterenol

		Ca content (mEq/kg wet wt)	
		Ca_1	Ca_2
Isoproterenol:	low dose (4)	0.17 ± 0.01[a]	2.87 ± 0.32
Isoproterenol:	low dose + Practolol	0.14 ± 0.01[b]	2.54 ± 0.32

Number in parenthesis indicates the number of hearts.

[a] Mean ± S.E.

[b] Significantly different from isoproterenol-treated hearts ($p < 0.05$).

SECTION IV.

DISCUSSION

I. The Effect of Hypoxia on Excitation-Contraction Coupling.

It is well established that a rapid reduction of myocardial contractility immediately follows an ischemic or hypoxic insult (e.g. Prinzmetal et al., 1949; Braasch, 1968; Dhalla et al., 1972). Since Ca probably represents the final common pathway for the coupling of excitation to contraction in the mammalian myocardium (Langer, 1965; Bassingthwaighte and Reuter, 1972; Morad and Goldman, 1973; Nayler and Seabra-Gomes, 1975), the reduction of myocardial contractility associated with hypoxia or ischemia may have occurred as a result of changes in myocardial Ca metabolism. Specific Ca pools in the myocardium have been implicated in the excitation-contraction coupling process by several investigators (Bailey and Dresel, 1968; Langer, 1973). Bailey and Dresel (1968) showed that the decay of developed tension during Ca-free perfusion was a monoexponential function of time. The magnitude and the rate of decay of contractile force was closely correlated respectively to the quantity of Ca contained in and the rate of washout of Ca from a single pool, Ca_{II}. The results obtained in this investigation suggest that the proposed one to one relationship between Ca_{II} and contractile activity may be oversimplified.

Graphical analysis of the curve describing the decay of dP/dt during Ca-free washout yielded two monoexponential components, $dP/dt_{(1)}$ and $dP/dt_{(2)}$. The halftime for the decay of $dP/dt_{(1)}$ was found in this study to be highly correlated to the halftime for the washout of Ca from Ca_{II} in normoxia as well as hypoxia just as reported by Bailey and Dresel (1968). The halftime for the decay of $dP/dt_{(2)}$ was also found to be significantly correlated to the washout of Ca from Ca_{III} in normoxia as

well as hypoxia. This suggests that Ca_{II} and Ca_{III}, and not a single pool, Ca_{II}, as reported by Bailey and Dresel (1968), are the fast and slow Ca pools respectively, required for the development and maintenance of contractile force in the heart. The discrepancy may be due to the use in this study of a different method for the measurement of contractile activity. Instead of estimating contractility by a clip connecting the apex of the heart to a force displacement transducer, intraventricular pressure and its derivative was measured by means of a saline-filled balloon inserted into the left ventricle. Since the balloon was inflated to give an end diastolic pressure of 5 mm Hg, the hearts had to perform isometric work by contracting against the load. Measurement of dP/dt by this method gave a more sensitive indication of contractile activity of the heart which may have been responsible for the inconsistency observed in the decay of contractile activity.

In agreement with many investigators, it was observed in the experiments described above that an immediate decline of overall dP/dt occurred when the heart was perfused with a solution containing a pO_2 of 41 mm Hg. Hypoxia had little effect on the second component of contractile activity, $dP/dt_{(2)}$, and thus the major reduction in contractility occurred in $dP/dt_{(1)}$, or that component requiring Ca from Ca_{II}. It was therefore not unexpected when it was observed that the Ca content of a pool, Ca_{II}, was reduced to 40% of control as early as 3 min after hypoxia. This reduction of Ca_{II} content was associated with a concomitant reduction of dP/dt to 64% which was maintained after 10 min of hypoxia. Ca_{II} which appears to be directly involved with the maintenance of contractile force in the heart (Bailey and Dresel, 1968), may represent Ca-released from

the sarcoplasmic reticulum. If this is so, these results agree with those of Schwartz et al., (1973) who have demonstrated that Ca release by the sarcoplasmic reticulum was impaired 12 min after the onset of ischemia. Schwartz and coworkers were unable to detect alterations in Ca binding or releasing capacity of other membranous organelles at this time. Schwartz believed that impairment of Ca release from the sarcoplasmic reticulum was responsible for contractile failure observed during early ischemia.

The content of Ca washed out of Ca_{III} was significantly increased after 3 min of hypoxia. The increase after 10 min of hypoxia was, however, not statistically significant. Indirect evidence suggests that Ca_{III} may represent Ca contained in cellular binding sites involved in Ca sequestration for muscular relaxation since Ca_{III} was not detected in the Ca-free washout of hearts which developed contracture after treatment with lanthanum (Bailey and Ong, 1974; Mezon and Bailey, 1975). Ca_{III} therefore may represent Ca contained in the mitochondria and the sarcoplasmic reticulum which have both been reported to be involved in the relaxation of cardiac muscle (Katz and Repke, 1967; Weber et al., 1967; Carafoli and Azzi, 1972; Lehninger, 1974).

Neither 3 nor 10 min hypoxic perfusion changed the total quantity of Ca washed out of the heart, that is, the sum of Ca_{II} and Ca_{III} contents. However, after 10 min of hypoxic perfusion, significantly more Ca remained in the tissue. The two-fold increase of residual Ca content indicates that hypoxia increased the quantity of Ca tightly bound in the heart. This tightly-bound Ca represents Ca not directly involved in excitation-contraction coupling since it was not readily exchangeable and remained in the tissue after 3 min of Ca-free washout when systolic LVP had decayed

to less than 5 mm of Hg. From direct determination of total tissue Ca, 10 min of hypoxia also resulted in a 27% increase of tissue Ca. Elevation of total tissue Ca in hypoxia or ischemia is well-documented (Shen and Jennings 1972; Lie et al., 1975). The subcellular location or the significance for accumulation of this relatively non-exchangeable Ca is still not known.

Nayler and Merrillees (1971) and Nayler et al., (1971) reported that the ability of cardiac muscle to accumulate Ca at superficial binding sites was reduced 10 min after the onset of hypoxia and 5 min afterischemia. This effect resulted in a decreased quantity of Ca displaced from these binding sites following depolarization. In agreement with these results, the present experiments have clearly shown that the Ca content of a superficial and small pool, Ca_1, was reduced from 0.271 to 0.088 mEq/kg tissue wet wt after 6 min of hypoxia. Ca_1 represents a labile and superficial Ca pool that may mediate the release of Ca from a second, larger and presumably intracellular Ca pool, Ca_{II} or Ca_2, directly involved in activating contraction (Bailey and Ong, 1974). It is likely that the content of Ca in Ca_1 may have been reduced earlier than 6 min after the onset of hypoxia. However, the quantity of Ca taken into Ca-depleted hearts was not determined until 6 min after the onset of hypoxia.

The quantity of Ca extracted by Ca-depleted hearts into Ca_2 was also reduced, although not significantly, after 6 min of hypoxia. Ca_2 represents, at least in part Ca contained in Ca_{II}, since ^{45}Ca specifically loaded into Ca_2 was subsequently recovered from Ca_{II} by Ca-free washout (Bailey et al., 1972). However, since the content of Ca in Ca_2 was found to be higher than that of Ca_{II}, Ca_2 from compartmental analysis probably

also includes part of Ca contained in Ca_{III}. Hence, changes in Ca_2 content may reflect sum of changes occurring in Ca_{II} and Ca_{III}. Thus even when Ca_{II} content was significantly reduced by hypoxia, the corresponding decrease of Ca_2 content may not be statistically significant.

In summary, hearts briefly exposed to hypoxic perfusion showed an immediate reduction in contractility possibly by reducing the content of Ca in two Ca pools: a small, superficial pool, Ca_1, and a larger, prabably intracellularly located pool, Ca_{II}. Hypoxia was also found to cause some increase of Ca_{III} content, probably representing a large Ca pool responsible for Ca binding for muscle relaxation. Although the total content of Ca washed out of the heart (total Wash II Ca) was not changed by hypoxia, there was a significant increase in the quantity of Ca remaining in the heart (residual tissue Ca) after 10 min of Ca-free washout. Residual tissue Ca represents tightly bound Ca not involved in the excitation-contraction coupling process. The significance for the marked elevation of this Ca compartment by hypoxia is still unknown at the present time.

II. The Effect of Practolol on Excitation-Contraction Coupling.

A. Normoxia.

Practolol at the dose used in this investigation (10^{-5}M) increased dP/dt by 28% in accord with other investigators (Dunlop and Shanks, 1968; Sowton et al., 1968). However no significant changes of Ca_{II} level were associated with the intrinsic activity of the drug. Practolol did not affect the total content of Ca washed out of the heart (total Wash II Ca) or the content of Ca remaining after Ca-free washout (residual tissue Ca). Both 3 and 10 min of treatment with practolol resulted in a reduction of

Ca_{III} content, although only the reduction after 3 min of treatment with practolol was statistically significant. Practolol reduced the quantity of Ca taken up by Ca_1. Hence, it has not been possible to attribute changes in Ca metabolism to explain the small positve inotropic effect of practolol. The inability to detect increase in Ca levels associated with increase of dP/dt may be due to tissue variability since Ca levels of control and treatment were not from the same animals (Comparison 3, see page 42 METHODS). Results obtained with isoproterenol have demonstrated a more than two-fold increase of Ca_{II} content associated with a 70-200% increase of dP/dt. If practolol exerted its effect on dP/dt by its intrinsic β_1 action, then it is possible that the content of Ca in Ca_{II} was also elevated by this drug, but these changes were not dectected in the present investigation.

B. Hypoxia.

Practolol prevented the reduction of Ca_{II} content caused by 3 min of hypoxia and diminished the reduction of Ca_{II} content caused by 10 min of hypoxia. Even in the presence of practolol, there was still a reduction of Ca_1 content after 6 min of hypoxic perfusion although the reduction was no longer statistically significant. Hence, practolol was able to prevent to some extent the distortions in Ca metabolism that may have been responsible for the reduction of contractility associated with hypoxia. It is therefore logical to expect that this drug should have some protective effect on the reduction of dP/dt caused by hypoxia. In contrast, the drug was found to be devoid of a protective effect on the reductions of contractile activity caused by both 3 or 10 min of hypoxia. Experiments on the restoration of dP/dt after hypoxia also

showed that practolol did not increase the rate of restoration of dP/dt upon reoxygenation after exposure to 3 or 10 min of hypoxia. The lack of protective effect of practolol on myocardial contractility during hypoxia is contradictory since the content of Ca washed out of the pools directly involved in E-C coupling was not significantly reduced after 3 or 10 min of hypoxia when practolol was also contained in the perfusate.

It is possible that the immediate reduction of contractile force during hypoxia is not related to changes in the content or kinetics of the Ca pools involved in E-C coupling. The reduction of dP/dt in hypoxia may be caused by a reduction of energy supply. Several investigators have reported reductions of high energy phosphate stores within ½ to 1 min after the onset of myocardial ischemia or hypoxia (Feinstein, 1962; Braasch et al., 1968; Scheuer and Stezoski, 1968; Dhalla et al., 1972). Hence, although practolol was able to prevent the reduction of Ca_{II} content after 3 min of hypoxia, it probably was unable to prevent the reduction of dP/dt due to deficiency of high energy phosphate stores. For the same reason, practolol was unable to increase the rate of restoration of dP/dt upon reoxygenation. Thus, contractility may have been compromised by at least two factors in hypoxia, changes in the Ca involved in excitation-contraction coupling and a reduction in the supply of energy for contraction. The latter mechanism must predominate since prevention of the distortions in Ca metabolism by practolol had no effect on contractility or its restoration in the hypoxic or immediately post-hypoxic myocardium.

The lack of effect of practolol on myocardial contractility during and subsequent to hypoxia also appears contradictory in view of

protective effect of this drug on the development of myocardial necrosis as a consequence of ischemia or hypoxia (Libby et al., 1973). However, Roba (1974) has shown that several other drugs such as verapamil, and a derivative, D600, as well as the β-blockers, propranolol and practolol which have been shown to protect against the development of cellular necrosis in ischemic myocardium are without beneficial effect on the hypoxia induced reduction of contractility. Moreover, several unrelated classes of drugs such as pronethalol, phentolamine, theophylline and procaine that are without effect on the development of myocardial ischemic injury protect against hypoxia induced reduction of contractility. One may conclude then that the protective effect of a drug against the development of myocardial necrosis induced by hypoxia or ischemia is not necessarily related to the ability of the drug to protect against the reduction of contractility. Indeed Katz (1973) suggested that the reduction of contractility after coronary occlusion may serve to conserve energy for the preservation of cellular integrity thereby delaying the development of contracture which not only is irreversible but impedes perfusion of viable, damaged tissue (Cooley et al., 1972).

The increase of Ca_{III} content after 3 or 10 min of hypoxia was no longer present after treatment with practolol. This suggests that the Ca-combining component of the sarcoplasmic reticulum and the mitochondria in hypoxia was restored to normal by practolol.

Practolol did not affect the total content of Ca washed out of the heart after 10 min of hypoxia. The increase of non-exchangeable tissue Ca observed after 10 min of hypoxia was not prevented after treatment with practolol. Thus, practolol was able to prevent some myocardial Ca accumu-

lation associated with hypoxia, that is, the increase of Ca_{III} content. However, the drug was unable to prevent the increase of residual tissue Ca caused by 10 min of hypoxic perfusion. Since the significance of the accumulation of residual tissue Ca and the reduction of Ca_{III} content in hypoxia was not established, it cannot be concluded that this is the mechanism of the protective effect of practolol during ischemia. However, one might speculate that the accumulation of Ca by the subcellular structures represented by Ca_{III} may in fact be the initial event leading to the ultimate death of the cell. Fleckenstein et al., (1974) suggested that "Ca overload" in the mitochondria is the cause of cellular necrosis after treatment with high doses of isoproterenol. Similar mitochondrial Ca overload was believed to occur during a hypoxic insult to the myocardium (Shen and Jennings, 1972a,b; Jennings and Ganote, 1974). According to Fleckenstein's hypothesis, increase accumulation of Ca causes the mitochondria to swell and to lose respiratory control and phosphorylating capacity which results in depletion of ATP and cell death. In subcellular fractionation studies, it has been observed that 10 min of hypoxia increased although not significantly, mitochondrial Ca content. Shen and Jennings (1972a, 1972b) have observed that myocardium reversibly injured by 10 min of ischemia followed by 20 min of arterial reflow did not actively accumulate Ca. However, 40 min of ischemia followed by 10 min of arterial reflow resulted in a significant increase of Ca uptake in the injured tissue and specifically in the mitochondria. Perhaps longer exposure to hypoxia may cause more mitochondrial Ca overload. There was, however no evidence from this investigation to support the statement.

III. The Effect of Isoproterenol on Excitation-Contraction Coupling.

A monoexponential decay of dP/dt was observed during Ca-free washout of hearts treated with isoproterenol, unlike the two monoexponential components observed in untreated hearts. The halftime of decay of dP/dt was correlated to the halftime for the washout of Ca from Ca_{II}. In control hearts, the contribution of Ca_{III} to $dP/dt_{(2)}$ was small and hence when halftime of washout of Ca_{II} and consequently decay of $dP/dt_{(1)}$ was increased by isoproterenol it became increasingly difficult to detect $dP/dt_{(2)}$.

The positive inotropic and chronotropic effects of isoproterenol are well documented (e.g., Innes and Nickerson, 1975; Kaufman et al., 1951). Part of the investigation with isoproterenol was designed to study the isoproterenol-induced changes in Ca metabolism that are associated with the positive inotropic effect of this drug. To study this effect, a dose of isoproterenol sufficient to cause a 70-100% increase of contractile force in each heart was infused into the perfusion medium. The dose of drug used in different experiments ranged from 2.4×10^{-8}M to 1.2×10^{-7}M. This concentration of isoproterenol was called the 'low dose' to distinguish it from a larger dose of drug used in a latter part of the investigation for the study of the initial events in the development of myocardial necrosis.

Treatment with a low dose of isoproterenol caused an increase of Ca_{II} content to 250% of control. Since Ca_{II} has been shown to be directly involved with the maintenance of contractile force in the heart (Bailey and Dresel, 1968), the inotropic effect of isoproterenol was thus found to be related to an increase of Ca_{II} content, probably representing Ca contained in the Ca-releasing component of the sarcoplasmic reticulum.

Ca-uptake experiments showed that a low dose of isoproterenol re-

sulted in a significant decrease of Ca taken up into Ca_1. Ca_1 probably represents a superficial, labile and small Ca pool which may mediate the release of Ca from a larger, intracellular pool, Ca_{II}, to initiate contraction (Bailey and Ong, 1974). Although the evidence is not conclusive, Ca_1 may represent the sources of Ca for the slow, inward Ca current described by a number of investigators (Tritthart et al., 1973a, 1973b; Bassingthwaighte and Reuter, 1972). It has been reported that β-adrenergic stimulants such as epinephrine and isoproterenol increase the slow inward Ca current and thereby increase contractile activity (Reuter, 1965; Fleckenstein et al., 1971; Tritthart et al., 1973b). However, an increase in Ca_1 level was not detected in hearts treated with isoproterenol. Since Ca_1 content in this study represents the capacity of a superficial pool to bind Ca whereas the slow Ca current represents the influx Ca associated with the individual action potential, it is conceivable that isoproterenol increased Ca current without simultaneously increasing the Ca_1 content. No explanation can be given for this possible discrepancy at this time.

Treatment of hearts with high concentrations of isoproterenol is known to produce myocardial necrosis (Bloom and Cancilla, 1969; Chappel et al., 1959; Rona et al., 1959; Fleckenstein et al., 1974) similar to that produced by ischemia or hypoxia (Rona et al., 1963; Niles et al., 1968). Some investigators believe that the necrosis produced by large doses of isoproterenol is caused by increased Ca influx and particularly increased accumulation of Ca by the mitochondria, i.e., "Ca overload" (Fleckenstein et al., 1974; Nirdlinger and Bramante, 1974). Mitochondria exposed to a high and low level of Ca have been known to actively sequester the ion (Legato et al., 1968; Hackenbrock and Caplan, 1969) at the expense

of energy production (Brierley, 1963; Lehninger, 1974) resulting in a deficiency of high energy phosphate stores. Deficiency of high energy stores could affect membrane stability, allowing still more Ca and Na to enter the cell. Accumulation of Ca in the mitochondria osmotically transform the mitochondria into the orthodox conformation which is normally related to resting respiration. However, in mitochondria overloaded with Ca, development of the orthodox conformation was associated with decrease in acceptor control and oxidative phosphorylation efficiency of the mitochondria. There was thus further reduction of high energy phosphate production by the mitochondria (Hackenbrock and Caplan, 1969). The ultimate consequence of this process is cell death. It was thus the goal of the second part of this investigation to study the effect of a high dose of isoproterenol on Ca metabolism and to compare the hypoxia induced anomalies of Ca metabolism with that produced by isoproterenol. The concentration of isoproterenol in the perfusate ranged from 1.3×10^{-7}M to 3.9×10^{-7}M and produced a 100-200% increase of dP/dt and invariably caused tachycardia and arrhythmias. Infusion of isoproterenol was continued for 25 min.

Treatment with the high dose of isoproterenol again caused an increase of Ca_{II} content to a level similar to that obtained by treatment with a low dose of the drug. The positive inotropic effect of this drug was thus related to the elevation of Ca_{II} content, but not in a linear fashion since a similar increase of Ca_{II} content was related to a higher increase of dP/dt.

The quantity of Ca washed out of Ca_{III} was significantly reduced

by a high dose of the drug. Since Ca_{III} probably represents the Ca-sequestering component of the sarcoplasmic reticulum and the mitochondria, these results suggest that the Ca accumulating capacity of these structures was reduced, unlike the effects observed by other investigators. To confirm these observations, the content of Ca in the mitochondria was determined directly and showed that 25 min of treatment with a high dose of isoproterenol caused a significant reduction of mitochondrial Ca content to 40% of control. Thus, the results of the experiments with isoproterenol appeared to conflict with results obtained by investigators who have shown that mitochondrial Ca was increased in hearts treated with high concentrations of isoproterenol (Bloom and Cancilla, 1969; Nirdlinger and Bramante, 1974). This discrepancy may be due to use by these investigators of a different experimental animal and a much higher dose of the drug. Nirdlinger and Bramante injected isoproterenol subcutaneously into rats at a dose of 40 mg/kg and observed an increase of mitochondrial Ca concentration together with transformation of the mitochondria into the orthodox conformation. Bloom and Cancilla injected isoproterenol intraperitoneally into rats at 5 mg/kg which resulted in mitochondrial calcification and focal myocytolysis.

The oxidized product of isoproterenol, an analogue to adrenochrome rather than isoproterenol has recently been shown to be the agent responsible for inducing myocardial necrosis (Yates and Dhalla, 1975). Care had been taken in this study to prevent the oxidation of isoproterenol prior to infusion. Perhaps this is another explanation why an increase of mitochondrial Ca content was not observed. The results obtained from a high dose of isoproterenol on Ca_{III} and mitochondrial Ca content were

thus different from those of hypoxia.

In practolol-treated hearts, the halftime for the washout of Ca from Ca_{II} was still increased in the presence of isoproterenol. Consequently, a monexponential decay of dP/dt was observed during Ca-free washout in isoproterenol-treated hearts in the presence of practolol.

Although practolol at 10^{-5}M blocked the positive inotropic effect of isoproterenol, the drug was unable to prevent significantly the increase of Ca_{II} content caused by a low dose of isoproterenol. This is consistent with the observation that high concentrations of propranolol (10^{-4}M) are required to cause a small reduction (9%) of maximal Ca-binding by isolated sarcoplasmic reticulum (Watanabe and Besch, 1974). Practolol did not affect the quantity of Ca washed out of Ca_{III}. Therefore, practolol had little effect on isoproterenol induced redistribution of Ca, from Ca_{III} to Ca_{II}.

Practolol was found to block the positive inotropic effect of isoproterenol perhaps by preventing the increase in the content of Ca in a small, superficial Ca pool, Ca_1 and thereby reducing Ca to be released from Ca_{II} to initiate contraction. It is also possible that practolol, like propranolol may also mediate its effect by blocking the isoproterenol induced inward Ca current (Watanabe and Besch, 1974). In fact, both mechanisms may be operating simultaneously to result in a blockade of inotropy caused by isoproterenol.

In summary, a reduction of the quantity of Ca contained in two specific Ca pools during early hypoxia was observed in this investigation namely, a small superficial pool Ca_1 and a larger intracellular pool Ca_{II}, possibly resulted in a reduction of contractility. Although practolol

hypoxia-induced changes in the Ca content of these pools, the drug did not prevent the reduction of contractility caused by hypoxia. The reason for the lack of effect of practolol on contractility may be due to the early reduction in the energy supply to support the contractile process.

The hypoxia induced increase of Ca_{III} content was also blocked by practolol. The drug however did not prevent the increase of residual tissue Ca caused by hypoxia. If the accumulation of Ca_{III} content represents partly an increase of Ca content of the mitochondria, blockade of this Ca overload by practolol may help to prevent subsequent damage to the mitochondria and the development of cellular necrosis associated with hypoxia.

Isoproterenol may have increased contractility by increasing the quantity of Ca in Ca_{II}. However, practolol did not prevent the isoproterenol induced increase of Ca_{II} content. This drug decreased the content of Ca in Ca_1 and by this mechanism may have prevented the increase of contractility caused by isoproterenol. Unlike the effect of hypoxia, isoproterenol was found to reduce both the content of Ca in Ca_{III} as well as the content of Ca contained in the mitochondria. The effect of isoproterenol on Ca metabolism is thus quite different from that of hypoxia. If the doses and duration of isoproterenol administration used in this experiment ultimately cause cellular damage, then early damage associated with isoproterenol is probably not mediated by an acute mitochondrial Ca overload. However, in experimentally induced isoproterenol necrosis produced by other investigations, the hearts were exposed to high concentrations of the drug for considerably longer periods of times. Thus, it cannot be conclusively stated on the basis of these results that mito-

chondrial Ca overload is not the mechanism causing cellular necrosis after large doses of isoproterenol.

SECTION V
BIBLIOGRAPHY

BIBLIOGRAPHY

Alpert, N.R. and Gordon, M.S. Myofibrillar adenosine triphosphatase activity in congestive heart failure. Am. J. Physiol. 202: 940-946, 1962.

Aronow, W.S. The medical treatment of angina pectoris VI. Propranolol as an antianginal drug. Am. Heart J. 84: 706-709, 1972.

Bailey, L.E. and Dresel, P.E. Correlation of contractile force with a calcium pool in the isolated cat heart. J. Gen. Physiol. 52: 969-982, 1968.

Bailey, L.E. and Ong, S.D. Calcium pools in excitation-contraction coupling in the cat heart. In Myocardial Biology, Vol. 4, edited by N.S. Dhalla, Baltimore, University Park Press, 1974, pp. 255-271.

Bailey, L.E., Ong, S.D. and Queen, G.M. Calcium movement during contraction in the cat heart. J. Mol. Cell. Cardiol. 4: 121-138, 1972.

Bailey, L.E. and Sures, H.A. The effect of ouabain on the washout and uptake of calcium in the isolated cat heart. J. Pharmacol. Exp. Therap. 178: 259-270, 1971.

Bárány, M., Gaetjens, E., Bárány, K. and Karp, E. Comparative studies of rabbit cardiac and skeletal myosins. Arch. Biochem. Biophys. 106: 280-293, 1964.

Barcroft, J. Anoxaemia. Lancet 2: 485-489, 1920.

Bassingthwaighte, J.B. and Reuter, H. Calcium movements and excitation-contraction coupling in cardiac cells. In Electrical Phenomena in the Heart, edited by W.C. De Mello, N.Y., Academic Press, 1972, pp. 353-395.

Becker, L.C., Fortuin, N.J. and Pitt, B. Effect of ischemia and antianginal drugs on the distribution of radioactive microspheres in the canine left ventricle. Circ. Res. 28: 263-269, 1971.

Beeler, G.W. Jr. and Reuter, H. Voltage clamp experiments on ventricular myocardial fibres. J. Physiol. 207: 165-190, 1970a.

Beeler, G.W. Jr. and Reuter, H. Membrane calcium current in ventricular myocardial fibres. J. Physiol. (Lond.) 207: 191-209, 1970b.

Beeler, G.W. Jr. and Reuter, H. The relation between membrane potential, membrane currents and activation of contraction in ventricular myocardial fibres. J. Physiol. (Lond.) 207: 211-229, 1970c.

Bellini, L. De urinis, et pulsibus, de missione sanguinis, de febribus, de morbis capitis, et pectoris. Bologna, Heirs of A. Pisarrius, 1683. Cited by Leibowitz, 1970, pp. 63-64.

Bettelheim, K. Ueber die Störungen der Herzmechanik nach Compression der Arteria coronaria sinistra des Herzens. Z. Klin. Med. 20: 436-443, 1892. Cited by Herrick, 1912.

Bloom, S., Brady, A.J. and Langer, G.A. Calcium metabolism and active tension in mechanically disaggregated heart muscle. J. Mol. Cell. Cardiol. 6: 137-147, 1974.

Bloom, S. and Cancilla, P.A. Myocytolysis and mitochondrial calcification in rat myocardium after low doses of isoproterenol. Am. J. Pathol. 54: 373-391, 1969.

Bloom, S. and Davis, D. Isoproterenol myocytolysis and myocardial calcium. In Myocardial Biology, Vol. 4, edited by N.S. Dhalla, Baltimore, University Park Press, 1974, pp. 581-590.

Braasch, W., Gudbjarnason, S., Puri, P.S., Ravens, K.G. and Bing, R.J. Early changes in energy metabolism in the myocardium following acute coronary artery occlusion in anesthetized dogs. Circ. Res. 23: 429-438, 1968.

Brierley, G.P. and Slautterback, D.B. Studies on ion transport IV. An electron microscope study of the accumulation of Ca^{2+} and inorganic phosphate by heart mitochondria. Biochim. Biophys. Acta 82: 183-186, 1964.

Brierley, G.P., Murer, E. and Green, D.E. Participation of an intermediate of oxidative phosphorylation in ion accumulation by mitochondria. Science 140: 60-62, 1963.

Brown, A., Aras, A. and Hass, G.M. Method for isolation of large quantities of human and canine cardiac myofibrils. J. Biol. Chem. 234: 438-440, 1959.

Carafoli, E. Mitochondria, Ca^{2+} transport and the regulation of heart contraction and metabolism. J. Mol. Cell. Cardiol. 7: 83-89, 1975.

Carafoli, E. and Azzi, A. The affinity of mitochondria for Ca^{++}. Experientia 27: 906-908, 1971.

Carsten, M.E. The cardiac calcium pump. Proc. Natl. Acad. Sci. U.S.A. 52: 1456-1462, 1964.

Chapman, R.A. and Niedergerke, R. Effects of calcium on the contraction of the hypodynamic frog heart. J. Physiol. (Lond.) 221: 389-421, 1972.

Chappel, C.I., Rona, G., Balazs, T. and Gaudry, R. Comparison of cardiotoxic actions of certain sympathomimetic amines. Can. J. Biochem. 37: 35-42, 1959.

Chirac, P. De motu cordis. Adversaria Analytica, 1698, pp. 121. Cited by W.T. Porter, 1894 and Miller, 1909.

Cohnheim, J. and Schulthess-rechberg, A.V. Ueber die Folgen der Kranzarterien-verschliessung für das Herz. Virchows Arch. Zellpathol 85: 503-507, 1881. Cited by Herrick, 1912 and Miller, 1909.

Cooley, D.A., Reul G. J. and Wukasch, D.C. Ischemic contracture of the heart: "Stone heart". Am. J. Cardiol. 29: 575-577, 1972.

Cornblath, M., Randle, P.J., Parmeggiani, A. and Morgan, H.E. Regulation of glycogenolysis in muscle. Effects of glucagon and anoxia on lactate production, glycogen content, and phosphorylase activity in the perfused isolated rat heart. J. Biol. Chem. 238: 1592-1597, 1963.

Cox, J.L., McLaughlin, V.W., Flowers, N.C. and Horan, L.G. The ischemic zone surrounding acute myocardial infarction. Its morphology as detected by dehydrogenase staining. Am. Heart J. 76: 650-659, 1968.

Degenring, F.H., Rubio, R. and Berne, R.M. Adenine nucleotide metabolism during cardiac hypertrophy and ischemia in rats. J. Mol. Cell. Cardiol. 7:105-113, 1975.

Dhalla, N.S., Yates, J.C., Walz, D.A., McDonald, V.A. and Olson, R.E. Correlation between changes in the endogenous energy stores and myocardial function due to hypoxia in the isolated perfused rat heart. Can. J. Physiol. Pharmacol. 50: 333-345, 1972.

Drelincourt, C. Cited by Leibowitz, 1970.

Dunlop, D. and Shanks, R.G. Selective blockade of adrenoceptive beta receptors in the heart. Br. J. Pharmacol. 32: 201-218, 1968.

Ebashi, S. and Endo, M. Calcium ion and muscle contraction. Prog. Biophys. Mol. Biol. 18: 123-183, 1968.

Ebbell, B. The Papyrus Ebers. The Greatest Egyptian Medical Document. Ejnar Munksgaard, Copenhagen, 1937, pp. 48.

Endo, M., Tanaka, M. and Ogawa, Y. Calcium induced release of calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibres. Nature 228: 34-36, 1970.

Entman, M.L., Levey, G.S. and Epstein, S.E. Mechanism of action of epinephrine and glucagon on the canine heart: evidence for increase in sarcotubular calcium stores mediated by cyclic 3', 5' -AMP. Circ. Res. 15: 429-438, 1969.

Erichsen, J.E. On the influence of the coronary circulation on the action of the heart. Cited by Porter, 1894.

Fabiato, A. and Fabiato, F. Excitation-contraction coupling of isolated cardiac fibres with disrupted or closed sarcolemmas. Ca-dependent cyclic and tonic contraction. Circ. Res. 31: 293-307, 1972.

Fabiato, A. and Fabiato, F. Contractions induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. J. Physiol. (Lond.) 249: 469-495, 1975.

Fawcett, D.W. and McNutt, N.S. The ultrastructure of the cat myocardium. I. Ventricular papillary muscle. J. Cell Biol. 42: 1-45, 1969.

Feinstein, M.B. Effects of experimental congestive heart failure, ouabain, and asphyxia on the high-energy phosphate and creatine content of the guinea pig heart. Circ. Res. 10: 333-346, 1962.

Fleckenstein, A. Specific inhibitors and promoters of calcium action in the excitation-contraction coupling of heart muscle and their role in the prevention or production of myocardial lesions. In Calcium and the Heart, edited by P. Harris and L.H. Opie, New York, Academic Press, 1971, pp. 135-188.

Fleckenstein, A., Janke, J., Döring, H.J. and Leder, O. Myocardial fiber necrosis due to intracellular Ca overlaod - new principle in cardiac pathophysiology. In Myocardial Biology, Vol. 4 edited by Dhalla, N.S., Baltimore, University Park Press, 1974, pp. 563-580.

Ford, L.E. and Podolsky, R.J. The regenerative calcium release within muscle cells. Science 167: 58-59, 1970.

Furchgott, R.F. and de Gubareff, T. The high energy phosphate content of cardiac muscle under various experimental conditions which alter contractile strength. J. Pharmacol. Exp. Ther. 124: 203-218, 1958.

Furchgott, R.F. and Lee, K.S. High energy phosphate and the force of contraction of cardiac muscle. Circulation 24: 416-428, 1961.

Gertz, E.W., Hess, M.L., Lain, R.F. and Briggs, F.N. Activity of the vesicular calcium pumping in the spontaneously failing heart-lung preparation. Circ. Res. 20: 477-484, 1967.

Gertz, E.W., Stam, A.C. Jr. and Sonnenblick, E.H. A quantitative and qualitative defect in the sarcoplasmic reticulum in the hereditary cardiomyopathy of the Syrian Hamster. Biochem. Biophys. Res. Commun. 40: 746-753, 1970.

Gomoll, A.W. and Braunwald, E. Comparative effects of sotalol and propranolol on myocardial contractility. Arch. Int. Pharmcodyn. Ther. 205: 338-354, 1973.

Green, C.W. and Gilbert, N.C. Blood pressure and electrocardiographic changes in the dog during extreme oxygen want. Am. J. Physiol. 55: 307-308, 1921.

Green, C.W. and Gilbert, N.C. Studies on the responses of the circulation to low oxygen tension. III. Changes in the pacemaker and in conduction during extreme oxygen want as shown in the human electrocardiogram. Arch. Intern. Med. 27: 517-557, 1921.

Greenawalt, J.W., Rossi, C.S. and Lehninger, A.L. Effect of active accumulation of calcium and phosphate ions on the structure of rat liver mitochondria. J. Cell Biol. 23: 21-38, 1964.

Gremels, H. and Starling, E.H. On the influence of hydrogen ion concentraction and of anoxaemia upon the heart volume. J. Physiol. 61: 297-304, 1926.

Hackenbrock, C.R. and Caplan, A.I. Ion-induced ultrastructural transformations in isolated mitochondria. The energized uptake of calcium. J. Cell Biol. 42: 221-234, 1969.

Harigaya, S. and Schwartz, A. Rate of calcium binding and uptake in normal animal and failing human cardiac muscle. Membrane vesicles (relaxing system) and mitochondria. Circ. Res. 25: 781-794, 1969.

Hasselbach, W., Fiehn, W., Makinose, M. and Migala, A.J. Calcium fluxes across isolated sarcoplasmic membranes in the presence and absence of ATP. In The Molecular Basis of Membrane function, edited by D.C. Tosteson, New Jersey, Prentice-Hall, Inc., 1969, pp. 299-316.

Hastings, A.B., Blumgart, H.L., Lowry, O.H. and Gilligan, D.R. Chemical changes in the heart following experimental temporary coronary occlusion. Trans. Assoc. Am. Physicians 54: 237-243, 1939.

Heberden, W. Cited by Leibowitz, 1970.

Heggtveit, H.A., Herman, L. and Mishra, R.K. Cardiac necrosis and calcification in experimental magnesium deficiency. A light and electron microscopic study. Am. J. Pathol. 45: 757-782, 1964.

Heggtveit, H.A. and Nadkarni, B.B. Ultrastructural pathology of the myocardium. Methods Achiev. Exp. Pathol. 5: 474-517, 1971.

Heilbrunn, L.V. and Wiercinski, F.J. The action of various cations on muscle protoplasm. J. Cell. Comp. Physiol. 29: 15-32, 1947.

Hellam, D.C. and Podolsky, R. J. Force measurements in skinned muscle fibres. J. Physiol. 200: 807-819, 1969.

Herrick, J. Clinical features of sudden obstruction of the coronary arteries. J.A.M.A. 59: 2015-2020, 1912.

Hess, M.L., Briggs, F.N., Shinebourne, E. and Hamer, J. Effect of adrenergic blocking agents on the calcium pump of the fragmented cardiac sarcoplasmic reticulum. Nature 220: 79-80, 1968.

Hirsch, C. and Spalteholz, W. Coronararterien und Herzmuskel Anatomische und experimentelle Untersuchungen. Dtsch. Med. Wochenschr. 33: 790, 1907. Cited by Miller, 1909.

Innes, I.R. and Nickerson, M. Norepinephrine, epinephrine, and the sympathomimetic amines. In The Pharmacological Basis of Therapeutics, 5th edition, edited by L.S. Goodman and A. Gilman, Toronto, Collier Macmillan Canada, Ltd., 1975, pp. 477-513.

Ito, Y. and Chidsey, C.A. Intracellular calcium and myocardial contractility IV. Distribution of calcium in the failing heart. J. Mol. Cell. Cardiol. 4: 507-517, 1972.

Ito, Y., Suko, J. and Chidsey, C.A. Intracellular calcium and myocardial contractility V. Calcium uptake of sarcoplasmic reticulum fractions in hypertrophied and failing rabbit hearts. J. Mol. Cell. Cardiol. 6: 237-247, 1974.

Jennings, R.B. Myocardial ischemia - observations, definitions and speculations. J. Mol. Cell. Cardiol. 1: 345-349, 1970.

Jennings, R.B. and Ganote, C.E. Structural changes in myocardium during acute ischemia. Circ. Res. 34 and 35: III-156 - III-172, 1974.

Jennings, R.B. and Reimer, K.A. Salvage of ischemic myocardium. Mod. Concepts Cardiovasc. Dis. 43: 125-130, 1974.

Katz, A.M. Effects of ischemia on contractile processes of heart muscle. Am. J. Cardiol. 32: 456-460, 1973.

Katz, A.M. Effects of ischemia on the cardiac contractile proteins. Cardiology 56: 276-283, 1971/72.

Katz, A.M. and Hecht, H.H. The early "pump" failure of the ischemic heart. Am. J. Med. 47: 497-502, 1969.

Katz, A.M. and Maxwell, J.B. Actin from heart muscle: sulfhydryl groups. Circ. Res. 14: 345-350, 1964.

Katz, A.M. and Repke, D.I. Quantitative aspects of dog cardiac microsomal calcium binding and calcium uptake. Circ. Res. 21: 153-162, 1967.

Katz, A.M., Repke, D.I., Tada, M. and Corkedale, S. Propranolol-induced inhibition of cardiac microsomal calcium-uptake, calcium-binding and epinephrine-stimulated adenylate cyclase. Cardiovasc. Res 8: 541-549, 1974.

Kaufman, J., Iglauer, A. and Herwitz, G.K. Effects of isuprel (isopropylepinephrine on circulation of normal man. Am. J. Med. 11: 442-447, 1951.

Keefer, C.S. and Resnik, W.H. Angina pectoris. A syndrome caused by anoxemia of the myocardium. Arch. Intern. Med. 41: 769-807, 1928.

Keelan, P. Double-blind trial of propranolol (Inderal) in angina pectoris. Br. Med. J. 1: 897-898, 1965.

Khan, M.I., Hamilton, J.T. and Manning, G.W. Protective effect of beta adrenoceptor blockade in experimental coronary occlusion in conscious dogs. Am. J. Cardiol. 30: 832-837, 1972.

Kim, N.D. and Harrison, C.E. $^{45}Ca^{++}$ accumulation by mitochondria and sarcoplasmic reticulum in chronic potassium depletion cardiomyopathy. In Recent Advances in Studies on Cardiac Structure and Metabolism, Vol. 4, edited by N.S. Dhalla, Baltimore, University Park Press, 1974, pp. 551-562.

Kohlhardt, M., Bauer, B., Krause, H. and Fleckenstein, A. Selective inhibition of the transmembrane Ca conductivity of mammalian myocardial fibres by Ni, Co and Mn ions. Pflügers Arch. 338: 115-123, 1973.

Krebs, H.A. and Henseleit, K. Untersuchunger Über die Harnstoffbildung im Tierkörper. Z. Physiol. Chem. 210: 33-66, 1932.

Langer, G.A. Calcium exchange in dog ventricular muscle: Relation to frequency of contraction and maintenance of contractility. Circ. Res. 17: 78-90, 1965.

Langer, G.A. Heart: Excitation-contraction coupling. Ann. Rev. Physiol. 35: 55-86, 1973.

Lee, K.S., Ladinsky, H. and Stuckey, J.H. Decreased Ca^{2+} uptake by sarcoplasmic reticulum after coronary artery occlusion for 60 and 90 minutes. Circ. Res. 21: 439-444, 1967.

Legato, M.J., Spiro, D. and Langer, G.A. Ultrastructural alterations produced in mammalian myocardium by variation in perfusate ionic composition. J. Cell. Biol. 37: 1-12, 1968.

Lehninger, A.L. Ca^{2+} Transport by mitochondria and its possible role in the cardiac contraction-relaxation cycle. Circ. Res. 34 and 35: III-83 - III-90, 1974.

Leibowitz, J.O. The History of Coronary Heart Disease. London, Wellcome Institute of the History of Medicine, 1970.

Lekven, J. Effect of practolol on left ventricular dimensions during coronary occlusion. Am. J. Cardiol. 36: 179-184, 1975.

Lertora, J.J.L., Mark, A.L., Johannsen, J., Wilson, W.R. and Abbaud, F.M. Selective beta-1 receptor blockade with oral practolol in man. J. Clin. Invest. 56: 719-724, 1975.

Levy, R.L., Barach, A.L. and Bruenn, H.G. Effects of induced oxygen want in patients with cardiac pain. Am. Heart J. 15: 187-200, 1938.

Libby, P., Maroko, P.R., Covell, J.W., Malloch, C.I., Ross, J. Jr. and Braunwald, E. Effect of practolol on the extent of myocardial ischaemic injury after experimental coronary occlusion and its effects on ventricular function in the normal and ischaemic heart. Cardiovasc. Res. 7: 167-173, 1973.

Lie, J.T. Histopathology of the conduction system in sudden death from coronary heart disease. Circulation 51: 446-452, 1975.

Lie, J.T., Pairolero, P.C., Holley, K.E., McCall, J.T., Thompson, H.K. and Titus, J.L. Time course and zonal variations of ischemia-induced myocardial cationic electrolyte derangements. Circulation 51: 860-866, 1975.

Lindenmayer, G.E., Harigaya, S., Bajusz, E. and Schwartz, A. Oxidative phosphorylation and calcium transport of mitochondria isolated from cardiomyopathic hamster hearts. J. Mol. Cell. Cardiol. 1: 249-259, 1970.

Long, A.R. Cardiovascular renal disease. Report of a case of three thousand years ago. Arch. Pathol. 12: 92-94, 1931.

Lowry, O.H., Krayer, O., Hastings, A.B. and Tucker, R.P. Effect of anoxemia on myocardium of isolated heart of the dog. Proc. Soc. Exp. Biol. Med. 49: 670-674, 1942.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275, 1951.

Luft, U.C. Irreversible Organveränderungen durch Hypoxämie im unterdruck. Beitr. Pathol. 98: 323-334, 1937.

Maroko, P.R., Braunwald, E., Covell, J.W. and Ross, J. Jr. Factors influencing the severity of myocardial ischemia following experimental coronary occlusion. Circulation 39 and 40: III-140, 1969.

Maroko, P.R., Kjekshus, J.K., Sobel, B.E., Watanabe, T., Covell, J.W., Ross, J. Jr. and Braunwald, E. Factors influencing infarct size following experimental coronary artery occlusions. Circulation 43: 67-82, 1971.

Maroko, P.R., Libby, P. and Braunwald, E. Effect of pharmacologic agents on the function of the ischemic heart. Am. J. Cardiol. 32: 930-936, 1973.

Maroko, P.R., Libby, P., Covell, J.W., Sobel, B.E., Ross, J. Jr. and Braunwald, E. Precordial S-T segment elevation mapping: an atraumatic method for assessing alterations in the extent of myocardial ischemic injury. The effects of pharmacologic and hemodynamic interventions. Am. J. Cardiol. 29: 223-230, 1972.

Mascher, D. Electrical and mechanical responses from ventricular muscle fibres after inactivation of sodium carrying system. Pflügers Arch. 317: 359-372, 1970.

Matlof, H.J., Bates, B.L. and Harrison, D.C. The hemodynamic effects of practolol in experimental myocardial infarction. Cardiology 58: 181-189, 1973.

McCollum, W.B., Crow, C., Harigaya, S., Bajusz, E. and Schwartz, A. Calcium binding by cardiac relaxing system isolated from myopathic Syrian hamsters (strains 14.6, 82.62 and 40.54) J. Mol. Cell. Cardiol. 1: 445-457, 1970.

Merkel, H. Zur Kenntnis der Kranzarterien des Menschlichen. Herzen. Verhandl. d. Deutsch. Path. Gesellsch., Jena, 1907, pp. 127. Cited by Miller, 1909.

Michal, G., Naegle, S., Danforth, W. H., Ballard, F.B. and Bing, R.J. Metabolic changes in heart muscle during anoxia. Am. J. Physiol. 197: 1147-1151, 1959.

Miller, J.L. and Matthews, S.A. Effect of the heart of experimental obstruction of the left coronary artery. Arch. Intern. Med. 3: 476-484, 1909.

Morad, M. and Goldman, Y. Excitation-contraction coupling in heart muscle: membrane control of development of tension. Prog. Biophys. Mol. Biol. 27: 257-313, 1973.

Muir, J.R., Dhalla, N.S., Orteza, J.M. and Olson, R.E. Energy-linked calcium transport in subcellular fractions of the failing rat heart. Circ. Res. 26: 429-438, 1970.

Nakamaru, Y. and Schwartz, A. Possible control of intracellular calcium metabolism by $[H^+]$: sarcoplasmic reticulum of skeletal and cardiac muscle. Biochem. Biophys. Res. Commun. 41: 830-836, 1970.

Nathan, D. and Beeler, G.W. Electrophysiologic correlates of the inotropic effects of isoproterenol in canine myocardium. J. Mol. Cell. Cardiol. 7: 1-15, 1975.

Nayler, W.G., Chipperfield, D. and Lowe, T.E. The negative inotropic effect of adrenergic beta-receptor blocking drugs on human heart muscle. Cardiovasc. Res. 3: 30-36, 1969.

Nayler, W.G., and Merrillees, N.C.R. Cellular exchange of calcium. In Calcium and the Heart, edited by P. Harris and L. Opie, New York, Academic Press, 1971, pp. 24-65.

Nayler, W.G. and Seabra-Gomes, R. Excitation-contraction coupling in cardiac muscle. Prog. Cardiovasc. Dis. 18: 75-88, 1975.

Nayler, W.G., Stone, J., Carson, V. and Chipperfield, D. Effect of ischemia on cardiac contractility and calcium exchangeability. J. Mol. Cell. Cardiol. 2: 125-143, 1971.

Niedergerke, R. Movements of Ca in frog heart ventricles at rest and during contractures. J. Physiol. (Lond.) 167: 515-550, 1963a.

Niedergerke, R. Movements of Ca in beating ventricles of the frog heart. J. Physiol. 167: 551-580, 1963b.

Niles, N.R., Zavin, J.D. and Morikado, R.N. Histochemical study of effects of hypoxia and isoproterenol on rat myocardium. Am. J. Cardiol. 22: 381-388, 1968.

Nirdlinger, E.L. and Bramante, P.O. Subcellular myocardial ionic shifts and mitochondrial alterations in the course of isoproterenol-induced cardiopathy of the rat. J. Mol. Cell. Cardiol. 6: 49-60, 1974.

Ochi, R. and Trautwein W. The dependence of cardiac contraction on depolarization and slow inward current. Pflügers Arch. 323: 187-203, 1971.

Opie, L.H. Substrate utilization and glycolysis in the heart. Cardiology 56: 2-21, 1971/72.

Parry, C.H. An inquiry into the symptoms and causes of the syncope anginosa, commonly called angina pectoris. London, 1799. Cited by W.T. Porter, 1894.

Pelides, L.J., Reid, D.S., Thomas, M. and Shillingford, J.P. Inhibition by β-blockade of the ST segment elevation after acute myocardial infarction in man. Cardiovasc. Res. 6: 295-301, 1972.

Pirzada, F.A., Hood, W.B., Messer, J.V. and Bing, O.H.L. Effects of hypoxia, cyanide and ischemia on myocardial contraction: observations in isolated muscle and intact heart. Cardiovasc. Res. 9: 38-46, 1975.

Porter, W.T. On the results of ligation of the coronary arteries. J. Physiol. 15: 121-138, 1894.

Porter, W.T. Ueber die Frage eines Coordinations-Centrum im Herz-Ventrikel. Pflügers Arch. 95: 366-371, 1893.

Pretorius, P.J., Pohl, W.G., Smithen, C.S. and Inesi, G. Structural and functional characterization of dog heart microsomes. Circ. Res. 25: 487-499, 1969.

Prinzmetal, M., Schwartz, L.L., Corday, E., Spritzler, R., Bergman, H.C. and Kruger, H.E. Studies on the coronary circulation. VI. Loss of myocardial contractility after coronary artery occlusion. Ann. Intern. Med. 31: 429-449, 1949.

Redo, S.F., and Porter, B.Y. The role of the lack of oxygen in irreversible cardiac arrest. Surg. Gynecol. Obstet. 109: 431-444, 1959.

Reimer, K.A., Rasmussen, M.M. and Jennings, R.B. Reduction by propranolol of myocardial necrosis following temporary coronary artery occlusion in dogs. Circ. Res. 33: 353-363, 1973.

Reuter, H. Über die Wirkung von Adrenalin auf den cellulären Ca-Umsatz des Meerschweinchenvorhofs. Naunyn-Schmiedeberg's Arch. Pharmacol. 251: 401-412, 1965.

Rich, T.L. and Langer, G.A. A comparison of excitation-contraction coupling in heart and skeletal muscle: An examination of "calcium-induced calcium-release." J. Mol. Cell. Cardial. 7: 747-765, 1975.

Riggs, D.S. The Mathematical Approach to Physiological Problems. The Williams and Wilkins Company, Baltimore, 1963.

Ringer, S. A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. J. Physiol. (Lond.) 4: 29-42, 1882-1883.

Roba, J. Protection by some β-adrenergic blocking agents and other cardiovascular drugs against acute anoxia in isolated guinea-pig atria. Arch. Int. Pharmacodyn. Ther. 211: 174-187, 1974.

Rona, G., Chappel, C.I., Balazs, T. and Gaudry, R. An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. Arch. Pathol. 67: 443-455, 1959.

Rona, G., Kahn, D.S. and Chappel, C.I. Studies on infarct-like myocardial necrosis produced by isoproterenol: A review. Rev. Can. Biol. 22: 241-255, 1963.

Sanborn, W.G. and Langer, G.A. Specific uncoupling of excitation and contraction in mammalian cardiac tissue by lanthanum. Kinetic studies. J. Gen. Physiol. 56: 191-217, 1970.

Sandow, A. Excitation-contraction coupling in skeletal muscle. Pharmacol. Rev. 17: 265-320, 1965.

Sayen, J.J., Sheldon, W.F., Peirce, G. and Kuo, P.T. Polarographic oxygen, the epicardial electro-cardiogram and muscle contraction in experimental acute regional ischemia of the left ventricle. Circ. Res. 6: 779-798, 1958.

Scales, B., McIntosh, D.A.D. Effects of propranolol and its optical isomers on the radiocalcium uptake and the adenosine triphosphatases of skeletal and cardiac sarcoplasmic reticulum fractions (SRF). J. Pharmacol. Exp. Ther. 160: 261-267, 1968.

Schelbert, H.R., Covell, J.W., Burns, J.W., Maroko, P.R. and Ross, J. Jr. Observations on factors affecting local forces in the left ventricular wall during acute myocardial ischemia. Circ. Res. 29: 306-316, 1971.

Scheuer, J. and Stezoski, S.W. Effects of high-energy phosphate depletion and repletion on the dynamics and electrocardiogram of isolated rat hearts. Circ. Res. 23: 519-530, 1968.

Schwartz, A. Calcium and the sarcoplasmic reticulum. In Calcium and the Heart, edited by P. Harris and L.H. Opie, New York, Academic Press, pp. 66-92, 1971.

Schwartz, A. Calcium metabolism. Cardiology 57: 16-23, 1972.

Schwartz, A. Electron transport and mitochondria. Cardiology 56: 35-42, 1971/72.

Schwartz, A., Lindenmayer, G.E. and Harigaya, S. Respiratory control and calcium transport in heart mitochondria from the cardiomyopathic Syrian hamster. Trans. N.Y. Acad. Sci. 30: 951-954, 1968.

Schwartz, A., Wood, J.M., Allen, J.C., Bornet, E.P., Entman, M.L., Goldstein, M.A., Sordahl, L.A., Suzuki, M. and Lewis, R.M. Biochemical and morphologic correlates of cardiac ischemia. Am. J. Cardiol. 32: 46-61, 1973.

Scott, J.E. Ion binding in solutions containing acid mucopolysaccharides. In Chemistry and Physiology of Mucopolysaccharides, edited by G. Quintarelli, Boston, Little-Brown, 1968, pp. 171-187.

Shen, A.C. and Jennings, R.B. Kinetics of calcium accumulation in acute myocardial ischemic injury. Am. J. Pathol. 67: 441-452, 1972b.

Shen, A.C. and Jennings, R.B. Myocardial calcium and magnesium in acute ischemic injury. Am. J. Pathol. 67: 17-440, 1972a.

Shinebourne, E., White, R. and Hamer, J. A qualitative distinction between the beta-receptor-blocking and local anesthetic actions of antiarrhythmic agents. Circ. Res. 24: 835-841, 1969.

Sommers, H.M. and Jennings, R.B. Ventricular fibrillation and myocardial necrosis after transient ischemia. Arch. Intern Med. 129: 780-789, 1972.

Sordahl, L.A., Johnson, C., Blailock, Z.R. and Schwartz, A. The mitochondrion. In Methods in Pharmacology, Vol. 1, edited by A. Schwartz, New York, Meredith Corp., 1971, pp. 247-286.

Sreter, F.A. Temperature, pH and seasonal dependence of Ca-uptake and ATPase activity of white and red muscle microsomes. Arch. Biochem. Biophys. 134: 25-33, 1969.

Stephen, S.A. Unwanted effects of propranolol. Am. J. Cardiol. 18: 463-468, 1966.

Suko, J., Vogel, J.H.K. and Chidsey, C.A. Intracellular calcium and myocardial contractility III. Reduced calcium uptake and ATPase of the sarcoplasmic reticular fraction prepared from chronically failing calf hearts. Circ. Res. 27: 235-247, 1970.

Sulakhe, P.V. and Dhalla, N.S. Excitation-contraction coupling in heart VII. Calcium accumulation in subcellular particles in congestive heart failure. J. Clin. Invest. 50: 1019-1027, 1971.

Tatooles, C.J. and Randall, W.C. Local ventricular bulging after acute coronary occlusion. Am. J. Physiol. 201: 451-456, 1961.

Tennant, R. and Wiggers, C.J. The effect of coronary occlusion on myocardial contraction. Am. J. Physiol. 112: 351-361, 1935.

Thebesius, A.C. Dissertatio Medica de Circulo Sanguinis in Corde. Cited by Leibowitz, 1970.

Thompson, M.E., McDonald, R.H., Leon, D.F. and Shaver, J.A. Specific beta adrenergic blockade by practolol. Clin. Pharmacol. Ther. 16: 750-760, 1974.

Tomlinson, C.W. and Dhalla, N.S. Excitation-contraction coupling in heart IX. Changes in the intracellular stores of calcium in failing hearts due to lack of substrate and oxygen. Cardiovasc. Res. 7: 470-476, 1973.

Tritthart, H., Kaufmann, R., Volkmer, H.P., Bayer, R. and Krause, H. Ca-movement controlling myocardial contractility I. Voltage-, current-, and time- dependence of mechanical activity under voltage clamp conditions (cat papillary muscles and trabeculae). Pflügers Arch. 338: 207-231, 1973.

Tyberg, J.V., Yeatman, L.A., Parmley, W.W., Urschel, C.W. and Sonnenblick, E.H. Effects of hypoxia on mechanics of cardiac contraction. Am. J. Physiol. 218: 1780-1788, 1970.

Vivian, S.R. and Bailey, L.E. An unbiased and automatic method of multi-component exponential curve peeling. To be submitted to Computers in Biomedical Research.

Wasserman, M.A. and Levy, B. Selective beta adrenergic receptor antagonism in the anesthetized dog. J. Pharmacol. Exp. Ther. 188: 357-367, 1974.

Watanabe, A.M. and Besch, H.R. Jr. Subcellular myocardial effects of verapamil and D_{600} : Comparison with propranolol. J. Pharmacol. Exp. Ther. 191: 241-251, 1974.

Webb, J.L. and Hollander, P.B. Metabolic aspects of the relationship between the contractility and membrane potentials of the rat atrium. Circ. Res. 4: 618-626, 1956.

Weber, A. and Herz, R. The relationship between caffeine contracture of intact muscle and the effect of caffeine on reticulum. J. Gen. Physiol. 52: 750-759, 1968.

Weber, A., Herz, R. and Reiss, I. On the mechanism of relaxing effect of fragmented sarcoplasmic reticulum. J. Gen. Physiol. 46: 679-702, 1963.

Weber, A., Herz, R. and Reiss, I. The nature of the cardiac relaxing factor. Biochem. Biophys. Acta. 131: 188-194, 1967.

Weissler, A.M., Kruger, F.A., Baba, N., Scarpelli, D.G., Leighton, R.F. and Gallimore, J.K. Role of anaerobic metabolism in the preservation of functional capacity and structure of anoxic myocardium. J. Clin. Invest. 47: 403-416, 1968.

White, R. and Shinebourne, E. The interaction of sympathetic stimulation and blockade on the calcium uptake of the sarcoplasmic reticulum. Cardiovasc. Res. 3: 245-248, 1969.

Wildenthal, K., Crie, J. and Vastagh, G. Cardiovascular function and survival during severe systemic hypoxaemia: influence of glucose-potassium-insulin solution and of beta-blockade. Circ. Res. 7: 174-180, 1973.

Williamson, J.R. Glycolytic control mechanism II. Kinetics of intermediate changes during the aerobic-anoxic transition in perfused rat heart. J. Biol. Chem. 241: 5026-5036, 1966.

Winegrad, S. The Possible role of Ca in excitation-contraction coupling of heart muscle. Circulation, 24: 523-529, 1961.

Yates, J.C. and Dhalla, N.S. Induction of necrosis and failure in the isolated perfused rat heart with oxidized isoproterenol. J. Mol. Cell. Cardiol. 7: 807-816, 1965.